



UNIVERSIDADE ESTADUAL DE CAMPINAS

INSTITUTO DE BIOLOGIA

**CAMILA FERNANDA DA SILVA**

**DISCOVERING NEW CAPITELLIDAE (ANNELIDA) FROM BRAZIL**

**DESCOBRINDO NOVOS CAPITELLIDAE (ANNELIDA) DO BRASIL**

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**CAMILA FERNANDA DA SILVA**

**DISCOVERING NEW CAPITELLIDAE (ANNELIDA) FROM BRAZIL**

**DESCOBRINDO NOVOS CAPITELLIDAE (ANNELIDA) DO BRASIL**

Tese apresentada ao Instituto de Biologia da Universidade Estadual de Campinas como parte dos requisitos exigidos para obtenção do Título de Doutora em Ecologia.

Thesis presented to the Institute of Biology of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Ecology.

Orientadora: Profa. Dra. Antonia Cecília Zacagnini Amaral

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não viveu, pode ser mais, mas  
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## RESUMO

Os Capitellidae estão entre os poliquetas comumente encontrados em fundos não consolidados, principalmente os enriquecidos organicamente. Atualmente, 44 gêneros são válidos, compreendendo 187 espécies. No Brasil há apenas o registro de 14 gêneros e 27 espécies. Os capitélídeos podem variar de formas muito pequenas e filiformes até robustas, possuem corpo dividido em tórax e abdômen, e não apresentam apêndices prostomiais ou peristomiais. Devido a uma morfologia homogênea, a taxonomia do grupo se torna difícil, acarretando em baixo conhecimento da família. Neste contexto, esse trabalho teve como objetivo o estudo da taxonomia morfológica e molecular dos capitélídeos, contribuindo assim, para o melhor conhecimento de sua biodiversidade e sistemática. O material de estudo é proveniente de 23 localidades diferentes, entre coletas avulsas e projetos temáticos realizados, em sua maioria, ao longo da costa brasileira: AMBES, BioPol-NE, BIOPORE, Biota-Araçá, HABITATS, PICS/FDP e Revizee, e na Dorsal de São Paulo, Margem Continental Brasileira, a Expedição Iata-Piuna. No total, 12 espécies de quatro gêneros (*Capitella*, *Heteromastus*, *Rashgua* e *Scyphoproctus*) foram examinadas, das quais 11 são consideradas novas para a ciência. Uma nova espécie de *Capitella*, encontrada em ossos de baleia da região abissal (4204 m) da Dorsal de São Paulo, foi descrita e sequências do gene 16S foram obtidas e utilizadas para comparações interespecíficas (Capítulo 1). Os resultados de análises morfológicas e moleculares (COI e 16S) revelaram a existência de quatro novas espécies, distintas de *C. capitata*, comparadas com espécimes coletados na localidade tipo. Evento que confirma a possibilidade de *C. capitata* não ocorrer na costa brasileira. Estas novas espécies estão distribuídas ao longo da costa, exceto uma delas, que foi registrada somente para a Baía do Araçá, São Sebastião (SP) (Capítulo 2). Ainda por meio de análises morfológicas, novas espécies dos gêneros *Heteromastus* (Capítulo 3), *Rashgua* (Capítulo 4) e *Scyphoproctus* (Capítulo 5) foram descritas.

## ABSTRACT

Polychaetes of the family Capitellidae are the most commonly found in soft-bottom habitats, mainly the organically enriched. Currently, forty-four genera are valid (comprising 187 species). In Brazil, just 14 genera and 27 species have been recorded. Capitellids range from very small to robust forms, their body is divided into thorax and abdomen, and they lack prostomial and peristomial appendages. Due to their homogenous morphology, the taxonomy of this group is very difficult, resulting in low knowledge of the family worldwide. Thus, the main aim of this work was the study of the taxonomy (molecular and morphological) of capitellids in order to contribute to the better knowledge of their biodiversity and systematics. The specimens were sampled from 23 different sites, including random collections and thematic projects carried out, mostly, along the Brazilian coast: AMBES, BioPol-NE, BIOPORE, Biota-Araçá, HABITATS, PICS / FDP and Revizee, and at the São Paulo Ridge, Brazilian Continental Margin, South Atlantic, the Iata-Piuna Expedition. In result, 12 species of four genera (*Capitella*, *Heteromastus*, *Rashgua* and *Scyphoproctus*) were examined, of which 11 are considered new to science. A new species of *Capitella*, found in whale bones from the abyssal São Paulo Ridge, (4204 m), was described and 16S sequences were obtained and used for interspecific comparisons (Chapter 1). The results of morphological and molecular analyzes (COI and 16S) revealed the existence of four new species, distinct from *C. capitata*, collected at the type locality. These results confirm the possibility that *C. capitata* does not occur along the Brazilian coast. One species occurs only at Araçá Bay (SP), while the others are distributed along the coast (Chapter 2). New species of genera *Heteromastus* (Chapter 3), *Rashgua* (Chapter 4) and *Scyphoproctus* (Chapter 5) have been described by morphological analyzes.

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## INTRODUÇÃO

A primeira espécie descrita da família Capitellidae foi *Capitella capitata* por Fabricius (1780), que a nominou primeiramente de *Lumbricus capitatus*. Posteriormente, Blainville (1828) alterou o nome para *Capitella capitata* e Grube (1862) foi quem criou a família Capitellidae agrupando, com *Capitella*, os táxons *Dasybranchus* e *Notomastus*. A primeira revisão da família foi feita por Eisig (1887) e posteriormente por Hartman (1947) e Fauchald (1977). Mais recentemente, Green (2002), estudando os Capitellidae do Mar de Andamão (Oceano Índico), fez uma revisão da morfologia da família, sugerindo novos caracteres para identificação das espécies, que têm sido adotados desde então. O trabalho referido acima contempla a redefinição de vários gêneros e descrição de um novo gênero e de 16 novas espécies.

Revisões em nível genérico também foram feitas. Warren (1976, 1991) revisou as espécies de *Capitella*, Hutchings & Rainer (1981) forneceram uma revisão da espécie tipo de *Heteromastus*, Ewing (1984) revisou os gêneros *Mastobbranchus* e *Peresiella*, Warren et al. (1994) revisaram as espécies de *Mediomastus*, García-Garza et al. (2009) redescreveram as espécies de *Notodasus* e o gênero *Notomastus* foi revisto por García-Garza et al. (2012).

Em relação à classificação da família, Fauchald (1977) propôs a criação da Ordem Capitellida, sugerindo associação dos Capitellidae com Maldanidae e Arenicolidae, embora nenhuma análise filogenética tenha sido feita para embasar esse agrupamento. A primeira filogenia da família baseada em caracteres morfológicos foi proposta por Rouse & Fauchald (1997). Os autores agruparam os capitélídeos com as famílias Cossuridae, Orbiniidae, Paraonidae, Scalibregmatidae, Opheliidae, Maldanidae e Arenicolidae para formar o clado Scolecida. Nielsen (1995) sugeriu que Clitellata e Capitellidae formam um grupo irmão e a justificativa dessa relação seria devido aos parapódios reduzidos, número restrito de órgãos reprodutivos e a ocorrência de hermafroditismo simultâneo em algumas espécies de *Capitella*. Esta proposta não foi suportada por nenhuma análise realizada por Rouse & Fauchald (1997), porém estes autores consideraram a família Capitellidae um grupo monofilético pela presença de ganchos multidentados com capuz.

A filogenia de Capitellidae incluindo dados moleculares também não avançou muito até o momento, no entanto, tem-se utilizado espécies desse grupo com o intuito de esclarecer as relações filogenéticas entre os “Polychaeta”. O primeiro resultado obtido foi a recuperação de *Capitella 'capitata'* como grupo irmão de *Protula* sp. (Serpulidae) (Winnepeninckx et al., 1995, 1998). Struck et al. (2002) mostrou que *C. 'capitata'* formou um clado com Sabellidae,

Serpulidae, Spionidae e Eunicida, porém com baixo suporte de ramo. Zrzavý et al. (2009) recuperaram o clado terebeloide-capiteloide, incluindo Terebelliformia, Arenicolidae-Maldanidae e Capitellidae-Echiurida, porém com baixo suporte dos dados morfológicos que foi sustentado somente pelo arranjo setal e presença de uma membrana gular. Embora as relações com outras famílias ainda sejam discutíveis, diversos autores recuperaram um clado monofilético, com alto suporte, de Capitellidae como sendo grupo irmão de Echiura (Brown et al., 1999; Bleidorn et al., 2003a,b; Colgan et al., 2006; Rousset et al., 2007; Struck et al., 2007; Goto, 2016). Algumas análises filogenéticas colocaram Capitellidae e Echiura como grupo irmão de Clitellata (Struck et al., 2011; 2015), porém é clara a necessidade de estudos adicionais para esclarecer tanto as relações dos Capitellidae com outros táxons, quanto as relações dentro da própria família.

Os capitelídeos são, em sua maioria, marinhos e ocorrem desde a zona entremarés até regiões abissais, vivendo em galerias formadas próximas à superfície do sedimento ou em tubos, que podem chegar até 15 cm de profundidade (Blake, 2000; Magalhães & Bailey-Brock, 2012; Silva et al., 2016). Eles estão entre os poliquetas mais comumente encontrados em sedimentos não consolidados, principalmente os enriquecidos organicamente, uma vez que diversas espécies do gênero *Capitella* são indicadoras de distúrbio ambiental (Grassle & Grassle, 1974; Pearson & Rosenberg, 1978; Reish, 1980). Alimentam-se usando sua faringe eversível e são classificados como comedores de depósito não seletivos (Fauchald & Jumars, 1979; Jumar et al., 2015). Os capitelídeos, que podem variar de formas muito pequenas e filiformes, com 1-2 mm de comprimento, até formas robustas, excedendo 30 cm, apresentam corpo dividido em tórax e abdômen e não possuem apêndices prostomiais ou peristomiais, conferindo-lhes uma aparência muito semelhante à de uma minhoca.

Embora os Capitellidae possam ser facilmente distinguíveis de outras famílias, sua identificação geralmente é difícil devido a sua morfologia homogênea. Consequentemente, há poucos caracteres externos distintivos em nível genérico e específico. Adicionalmente, a definição dos gêneros é controversa, baseada em grande parte no arranjo dos setígeros torácicos (número de setígeros e distribuição dos diferentes tipos de cerdas), que provou ser variável com a idade e maturidade sexual (Ewing, 1982, 1984; Blake, 2000). Como consequência desse impasse taxonômico do grupo, uma generalização nas identificações das espécies acaba ocorrendo e acarretando na ampliação da distribuição das mesmas, conhecida como “síndrome cosmopolita” (Fauchald, 1984; Hutchings & Glasby, 1991).



Apesar da existência de espécies totalmente crípticas, que a morfologia por si só não consegue detectar, há muitos casos de espécies pseudo-crípticas, as quais as diferenças morfológicas podem ser detectadas após um exame mais detalhado dos espécimes (Nygren et al., 2009; Jörger & Schrödl, 2013). Revisões morfológicas mais acuradas, juntamente com técnicas moleculares, têm auxiliado no levantamento da real diversidade local de várias espécies e restringindo sua distribuição geográfica (Barroso et al., 2010; Nygren & Pleijel, 2011; Capa et al., 2013; Magalhães et al., 2014; Reish et al., 2014; Sun et al., 2016).

Um exemplo clássico entre os Capitellidae é o complexo *Capitella capitata*. Esta espécie foi originalmente descrita na Groenlândia, mas os registros de sua ocorrência foram mais tarde expandidos para todos os oceanos (Eisig, 1887; Hartman, 1947; Warren, 1976; Blake, 2009). De acordo com um estudo sobre sua redescrição e biologia, Blake (2009) concluiu que *C. capitata* ocorre apenas no Círculo Polar Ártico e que os registros fora desse limite deveriam ser revisados, pois, provavelmente, muitas espécies estão sendo subestimadas taxonômica e ecologicamente pelas identificações generalizadas.

Apesar dos capitélídeos serem muito abundantes em amostras de ambientes de fundos não consolidados areno-lamosos e relativamente fáceis de serem coletados, sua diversidade é pouco estudada. Comparado ao número total de táxons válidos atualmente, 44 gêneros e 188 espécies (Read, 2017), o registro de Capitellidae para o Brasil, contudo, é baixo, 12 gêneros e 24 espécies (Amaral et al., 2013). Além do mais, estes números são referentes a trabalhos de cunho ecológico, sendo apenas três os realizados com objetivo estudar a taxonomia e, consequentemente, a diversidade desta família (Amaral, 1980; Silva et al., 2016, 2017).

Com base no panorama apresentado, esta tese teve como objetivo o estudo da taxonomia morfológica e molecular da família Capitellidae, contribuindo assim, para o melhor conhecimento de sua biodiversidade e sistemática. Especificamente, este trabalho se propôs a:

- estudar a taxonomia morfológica, destacando os principais caracteres utilizados para cada gênero, com elaboração de pranchas ilustrativas, tabelas comparativas e chaves de identificação;
- analisar a taxonomia molecular dos espécimes, quando a extração de DNA fosse possível, confrontando as sequências obtidas com as disponíveis, para estudos comparativos intra e interespecíficos, bem como para construção de árvores filogenéticas;
- alimentar bancos de dados genéticos para uso em futuros estudos tanto moleculares, como morfológicos e filogeográficos, disponibilizando as sequências obtidas;

- identificar, molecular e morfológicamente, espécimes de *Capitella*, provenientes de ossos de baleia de região abissal, habitat até então sem registro de ocorrência de Capitellidae; e

- investigar a distribuição e o número de espécies que compõe as populações do complexo *Capitella capitata* ao longo da costa brasileira, caracterizando-as molecular e morfológicamente.

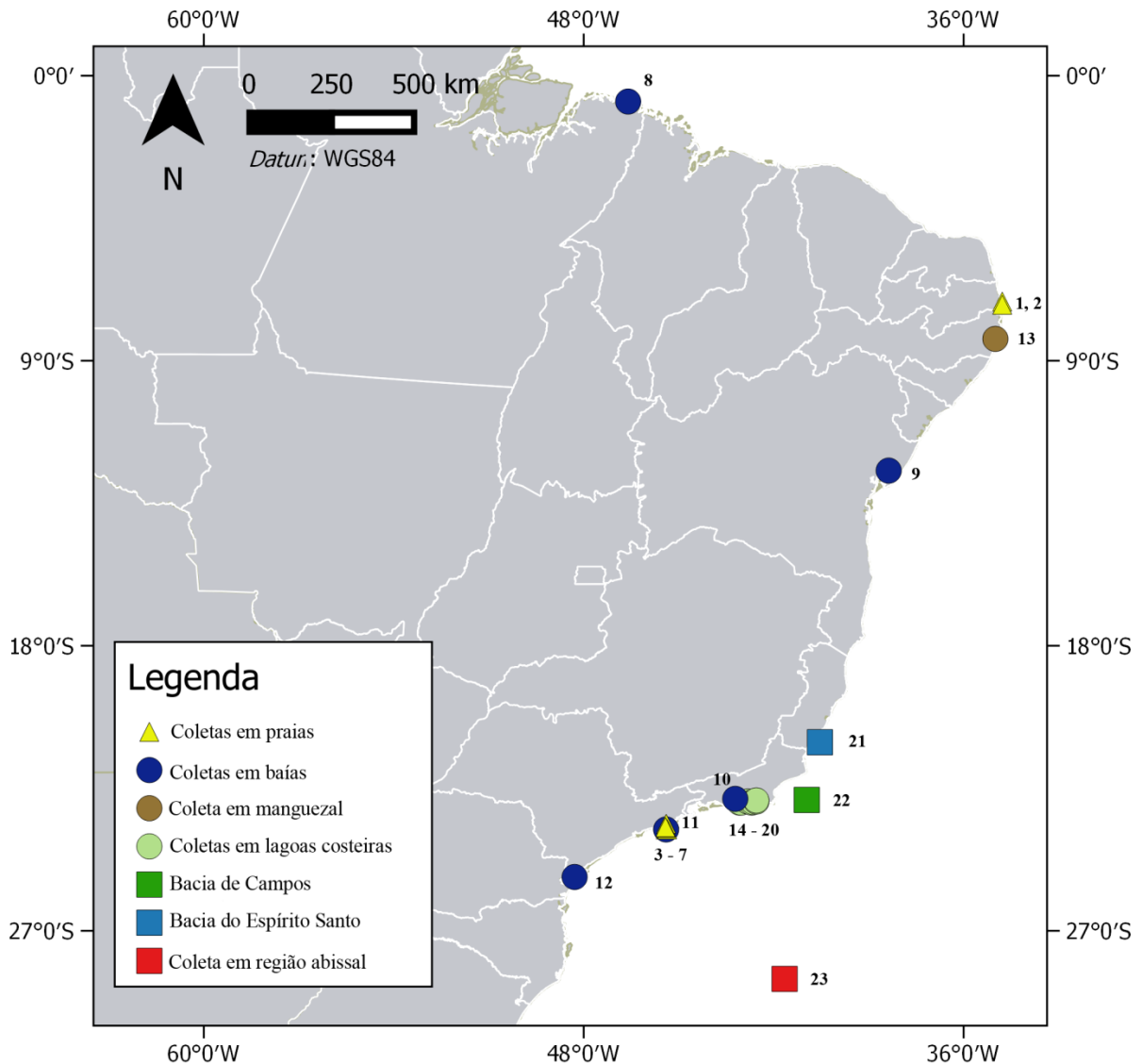
A partir dos objetivos propostos, a tese foi estruturada em cinco capítulos e considerações finais. Cada capítulo buscou investigar um gênero e, quando houve mais de um capítulo com o mesmo gênero, eles apresentaram diferentes objetivos. Assim, o capítulo 1 aborda a descoberta de uma nova espécie de *Capitella*, encontrada em ossos de baleia da região abissal (4204 m) da Dorsal de São Paulo, Margem Continental Brasileira, que foi descrita e sequências do gene 16S obtidas e utilizadas para comparações interespecíficas. Com o propósito de estudar o complexo *Capitella capitata*, o capítulo 2 analisa a taxonomia morfológica e molecular (genes COI e 16S) de quatro espécies de *Capitella*, bem como sua distribuição ao longo da costa brasileira corroborando resultados anteriores, que limitaram a ocorrência de *C. capitata* ao Círculo Polar Ártico, apenas. Dando continuidade ao estudo dos Capitellidae para o Brasil, os capítulos seguintes abordaram a taxonomia morfológica do material obtido em diferentes habitats ao longo da costa, resultando em descrições de novas espécies dos gêneros *Heteromastus* (Capítulo 3), *Rashgua* (Capítulo 4) e *Scyphoproctus* (Capítulo 5).

## ÁREAS DE ESTUDO

O material de estudo é proveniente de 23 localidades entre coletas avulsas e projetos temáticos realizados, em sua maioria, ao longo da costa brasileira e na região abissal da Margem Continental Brasileira (Fig. 1). As coletas foram realizadas em diversos habitats, sendo eles praias, planície de maré, sublitoral raso até região abissal e ambientes específicos, manguezais e banco de mexilhões, conforme descrições a seguir.

- 1) PICS/FDP - "Projeto Integrado do Canal de São Sebastião/Fauna de Praia" (1995 - 1997) ( $23^{\circ}43'$  -  $23^{\circ}49'S$  /  $45^{\circ}27'$  -  $45^{\circ}24'W$ ), com objetivo de estudar a macrofauna de 13 praias ao longo do Canal de São Sebastião, São Paulo, Sudeste do Brasil;
- 2) REVIZEE/Score Sul - "Programa de Avaliação dos Recursos Vivos da Zona Econômica Exclusiva" (1997 - 1998) ( $21^{\circ}$  -  $34^{\circ}S$  /  $40^{\circ}$  -  $52^{\circ}W$ ), com foco no conhecimento da biodiversidade e avaliação do potencial sustentável dos recursos faunísticos da Zona Econômica Exclusiva (ZEE) brasileira. As coletas foram realizadas na Plataforma Continental e Talude (60 - 800 m), entre os estados do Rio de Janeiro e Rio Grande do Sul, Sudeste e Sul do Brasil (ver detalhes em Amaral et al., 2004);
- 3) BIOPORE "Biologia Populacional e Reprodutiva de Invertebrados" (2006 - 2007) ( $23^{\circ}37'S$  -  $45^{\circ}23'W$ ), com o propósito de estudar a biologia reprodutiva e populacional de invertebrados que habitam bancos de mexilhão de fundo não consolidado de praias da Enseada de Caraguatatuba, São Paulo, Sudeste do Brasil (ver detalhes em Silva, 2013);
- 4) HABITATS / CENPES / PETROBRAS - "Heterogeneidade Ambiental da Bacia de Campos" (2008 - 2009) ( $21^{\circ}$  -  $24^{\circ}S$  /  $38^{\circ}$  -  $45^{\circ}W$ ), com o objetivo de pesquisar quatro habitats de fundo não consolidado (12 - 3301 m): foz do rio Paraíba do Sul, Plataforma Continental, Talude e cânions Almirante Câmara (CANAC) e Grussaí (CANG), Rio de Janeiro e Espírito Santo, Sudeste do Brasil (ver detalhes em Lavrado & Brasil, 2010);
- 5) BIOPOL-NE - "Diversidade de Polychaeta (Annelida) de Substratos Consolidados no Nordeste do Brasil" (2009 - 2010) ( $6^{\circ}$  -  $8^{\circ}S$  /  $34^{\circ}$  -  $35^{\circ}W$ ), estudo com foco em costões rochosos, tufo de algas, bancos de mexilhões, colônias de esponjas e ascídias, fragmentos de recifes de sabelariídeos e substratos similares, ao longo de praias dos

- estados da Paraíba e Pernambuco, Nordeste do Brasil (ver detalhes em Paresque, 2014);
- 6) AMBES – "Caracterização Ambiental da Bacia do Espírito Santo" (2010 – 2013) ( $18^{\circ}$  -  $21^{\circ}$ S /  $37^{\circ}$  -  $40^{\circ}$ W), com o objetivo de pesquisar quatro habitats de fundo não consolidado (25 – 3000 m): foz do Rio Doce, Plataforma Continental, Talude e cânions Watu Norte (CANWN) e Rio Doce (CAND), Espírito Santo, Sudeste do Brasil;
  - 7) EXPEDIÇÃO IATA-PIUNA - Parte de uma parceria entre o Brasil e Japão, durante a viagem ao redor do mundo "Quest for the Limit of Life" (2013 – 2014), pesquisa com objetivo de estudar o assoalho oceânico. Amostras realizadas com mergulhos do HOV Shinkai-6500, na Dorsal de São Paulo, Margem Continental Brasileira, Atlântico Sul (ver detalhes em Sumida et al., 2016);
  - 8) OIL SPILL - "Monitoramento derramamento de óleo" (2013 – 2014) ( $23^{\circ}48'S$  -  $45^{\circ}4'W$ ), com objetivo de monitorar a macrofauna de fundos não consolidados de praias arenosas afetadas por um acidente de derramamento de óleo no Canal de São Sebastião, costa norte do Estado de São Paulo, Sudeste do Brasil;
  - 9) BIOTA/FAPESP – Araçá, "Biodiversidade e funcionamento de um ecossistema costeiro subtropical: subsídios para gestão integrada" (2012 – 2017) ( $23^{\circ}48'S$  -  $45^{\circ}4'W$ ), projeto com objetivo de estudar a fauna de diferentes habitats (0 – 25 m): manguezal, planície de maré e sublitoral da Baía do Araçá, Canal de São Sebastião, costa norte do estado de São Paulo, Sudeste do Brasil (ver detalhes em Amaral et al., 2016).



**Fig 1. Distribuição dos locais de coletas onde foram amostrados os Capitellidae.** Coletas em praias: 1. Praia Cabo Branco, PB; 2. Praia Jacumã, PB; 3. Praia do Camaroeiro, SP; 4. Praia da Cidade, SP; 5. Praia Engenho D'Água, SP; 6. Praia São Francisco, SP; 7. Praia da Enseada, SP. Coletas em baías: 8. Baía Caetê, PA; 9. Baía de Todos os Santos, BA; 10. Baía de Guanabara, RJ; 11. Baía do Araçá, SP; 12. Baía de Paranaguá, PR; Coleta em manguezal: 11. Baía do Araçá, SP; 13. Maracaípe, PE; Coletas em lagoas costeiras: 14. Piratininga, RJ; 15. Itaipú, RJ; 16. Maricá, RJ; 17. Guarapina, RJ; 18. Jaconé, RJ; 19. Saquarema, RJ; 20. Araruama, RJ. Coleta em bacias: 21. Bacia do Espírito Santo, ES; 22. Bacia de Campos (ES e RJ). Coleta em região abissal: 23. Dorsal de São Paulo, Margem Continental Brasileira.

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## CAPÍTULO 1

### **A new *Capitella* polychaete worm (Annelida: Capitellidae) living inside whale bones in the abyssal South Atlantic**

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# A new *Capitella* polychaete worm (Annelida: Capitellidae) living inside whale bones in the abyssal South Atlantic



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## ABSTRACT

A new species of the genus *Capitella*, *Capitella iatapiuna* sp. nov., has been found in deep sea whale-fall samples, São Paulo Ridge-Southwest Atlantic. The new species is mainly characterized by a bluntly rounded prostomium and a very distinct peristomium forming a complete ring. Ribosomal 16S sequences were obtained and used for inter-specific comparisons. This species is herein described and compared to others species of the genus. Its ecological role in the whale-fall community is also discussed.

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## 1. Introduction

Sunken whale carcasses are the largest organic inputs in the deep-sea, which can sustain an invertebrate community for many years during at least three overlapping successional stages (Smith and Baco, 2003; Braby et al., 2007; Fujiwara et al., 2007; Lundsten et al., 2010a; Smith et al., 2015). After the consumption of all flesh tissue by scavengers, a dense heterotrophic community of invertebrates and microorganisms colonizes and degrades the lipid-rich bones and the organic-impacted sediments (Smith and Baco, 2003). These processes decrease the oxygen concentration in sediments immediately below and surrounding the carcass leading to increases in reduced compounds fluxes (i.e., sulfide and methane) mainly by the activity of anaerobic microorganisms (Deming et al., 1997; Goffredi et al., 2008; Treude et al., 2009). This is known as the sulfophilic stage, which is a harsh environment for many organisms.

Polychaetes represent ca. 60% of the total species richness in all successional stages of whale-falls communities (Smith and Baco, 2003). They are considered opportunistic organisms that tolerate high sulfide concentration levels (Smith et al., 1998a). Many polychaetes take advantage of the dissolved organic matter

available (Dahlgren et al., 2004) and/or actively feed on microbial mats both in whale falls and other cognate communities (Glover et al., 2005; Wiklund et al., 2009).

Even though different polychaete genera inhabit deep-sea whale-fall communities, *Capitella* was only recently reported for deep-sea implanted whalebones (Amon et al., 2015). Nevertheless, *Capitella perarmata* (Gravier, 1911) and *Capitella capitata* (Fabricius, 1780) were found in shallow-water whalebones from Antarctica (Taboada et al., 2013, 2015a) and the Mediterranean Sea (Taboada et al., 2015b), respectively. Both species are very common in organically enriched sediments (Warren, 1976; Conlan et al., 2004), but compared to other polychaetes they were found in very low densities inside whalebones (Taboada et al., 2013, 2015a,b). On the other hand, in the deepest whale-fall community reported to date (4204 m depth) (Sumida et al., in preparation), *Capitella iatapiuna* sp. nov. is one of the most abundant polychaetes inhabiting bones (Alfaro-Lucas et al., in preparation).

*Capitella* is the best known opportunistic polychaete genus in organically enriched sediments (Blake et al., 2009). For a long time its taxonomy was neglected (Green, 2002) owing to the relatively few distinct morphological characters that can be used for classification (García-Garza and León-González, 2011). Only recently some taxonomic reviews (Green, 2002; Blake, 2009; Blake et al., 2009; Magalhães and Bailey-Brock, 2012) and molecular data (Silva et al., in preparation) have been carried out. Despite the morphological simplicity of *Capitella*, details of the prostomium/

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peristomium, shape, size, and form of the thoracic segments, details of the genital spines, numbers of capillaries and hooks per setal fascicle, presence/absence of dorsal and lateral grooves, and methyl green staining patterns can all be used together with overall size and segment counts to define individual species (Blake, 2009).

Here we describe a new *Capitella* species on the basis of morphological analyses as well as mitochondrial (16S) gene and summarize the diagnostic characteristics of all valid species in a comparative table. We also discuss its ecological role on the abyssal Southwest Atlantic whale-fall communities.

## 2. Material and methods

The specimens were collected inside whale bones (Fig. 2A) located at the base of São Paulo Ridge (28° 31.1191' S 41° 39.4097' W) in the Southwest Atlantic Ocean (4204 m depth). Sampling was carried out during two dives of the HOV Shinkai-6500 as part of the Iata-Piuna Expedition, a partnership between Brazil and Japan, during the around the world voyage 'Quest for the Limit of Life' – QUELLE-2013 on board the R/V 'Yokosuka' (JAMSTEC). The whale carcass was discovered in an area characterized by few centimeters of soft-sediments overlying basalts rocks. This area is constantly influenced by the Antarctic Bottom Water (AABW) (Speer and Zenk, 1993).

Polychaetes were preserved in 96% ethanol. Measurements were made using optical microscopy and stereomicroscopy. Body length was measured from the anterior margin of the prostomium to the tip of the pygidium or to the posterior end of the available fragments. Width was measured at the widest segment (5th setiger), excluding chaetae. The specimens were examined under a stereomicroscope and images were recorded with a digital camera. For scanning electron microscope (SEM) images, specimens were dehydrated in a series of ethanol solutions with progressively increasing concentrations (75–100%), critical-point-dried with a Balzers CPD 30 (temperature 37 °C and pressure 70 kg/cm<sup>2</sup>), covered with a layer of 10–20 nm of gold. After treatment, specimens were observed under a JEOL JSM-5800 LV SEM at Laboratório de Microscopia Eletrônica, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP). The material was deposited at the Museu de Zoologia, Universidade Estadual de Campinas (ZUEC) and the Museu de Zoologia, Universidade de São Paulo (MZUSP).

Genomic DNA of six specimens was extracted with a DNeasy Blood & Tissue Kit (QIAGEN). A fragment of the mitochondrial genes cytochrome oxidase subunit 1 (COI) and of the 16S were amplified using universal primers, dglCO1490/dgHCO2198 (Meyer, 2003) and 16Sar-L/16Sbr-H (Palumbi et al., 1991), respectively. PCR reactions consisted of PuReTaq Ready-To-Go™ PCR Beads (GE Healthcare), 1.5 µL of each primer, 2 µL of DNA and 20 µL of water. The thermal cycling conditions were one cycle of 94 °C for 3 min, 5 cycles of 94 °C for 30 s, 42 °C for 40 s and 72 °C for 90 s, 32 cycles of 94 °C for 30 s, 46 °C for 40 s and 72 °C for 90 s, followed by a final extension step of 72 °C for 7 min. PCR products were purified and sequenced by Macrogen Inc. Electropherograms were edited with Sequencher 4.1 (Gene Codes Corporation) and sequences were aligned in Mega 5.0 (Tamura et al., 2011) with ClustalW tool. These new sequences were deposited in GenBank database ([www.ncbi.nlm.nih.gov/genbank](http://www.ncbi.nlm.nih.gov/genbank)) under the accession numbers (KU160141 and KU160142).

Pairwise genetic distance was estimated using Kimura's two-parameters (K2P) method (Nei and Kumar, 2000) in Mega 5.0 (Tamura et al., 2011). For inter-specific comparisons *Capitella teleta* Blake et al. (2009) a 16S sequence was taken from GenBank (accession number JF 509722).

## 3. Results

### Taxonomy

#### Family Capitellidae Grube, 1862

#### Genus *Capitella* Blainville, 1828

Type species, *C. capitata* (Fabricius, 1780) as *Lumbricus capitatus*. Redescribed by Blake (2009).

Type locality. West Greenland.

**Diagnosis** (emended by Magalhães and Bailey-Brock (2012)).

Prostomium conical to bluntly rounded, sometimes dorsoventrally flattened, with dorsal groove present or absent, with nuchal organs as paired slits at border between prostomium and peristomium; eyes present or absent. Peristomium forming a complete or incomplete asetigerous ring. Thorax with nine segments, all setigerous with capillary setae in both rami of setigers 1–3, 1–4, 1–6, or 1–7, otherwise with capillaries and hooks in various combinations in both rami; setigers 8–9 with hooded hooks, mixed capillaries and hooks, or all capillaries, these arrangements sometimes growth dependent; prominent genital spines present in setigers 8–9 of males and hermaphrodites; females usually with enlarged lateral genital pores between setigers 7–8 or 8–9. Capillaries unilimbate, with narrow wings; hooded hooks with multiple rows of denticles above main fang. Abdominal segments with hooded hooks in both rami; capillaries absent. Branchiae present or absent. Pygidium without appendages.

#### *Capitella iatapiuna* sp. nov.

**Type material.** All material collected at São Paulo Ridge, Southwest Atlantic. Holotype: ZUEC POL 16780–28° 31.1191' S 41° 39.4097' W, est. 1334–103; 4204 m deep, col. 24 April 2013, 1 spec. Paratypes: ZUEC POL 16781–28° 31.1191' S 41° 39.4097' W, est. 1334–103; 4204 m deep, col. 24 April 2013, 1 spec.; MZUSP 2743–28° 31.1191' S 41° 39.4097' W, est. 1334–103; 4204 m deep, col. 24 April 2013, 1 spec.; MZUSP 2744–28° 31.1191' S 41° 39.4097' W, est. 1334–103; 4204 m deep, col. 24 April 2013, 1 spec.

**Additional material examined.** ZUEC POL 16782–28° 31.1191' S 41° 39.4097' W, est. 1334–103; 4204 m deep, col. 24 April 2013, 11 specs.; ZUEC POL 16783–28° 31.1191' S 41° 39.4097' W, est. 1336–103; 4204 m deep, col. 24 April 2013, 4 specs.; ZUEC POL 16784–28° 31.1191' S 41° 39.4097' W, est. 1334–103; 4204 m deep, col. 24 April 2013, 5 specs.; ZUEC POL 16785–28° 31.1191' S 41° 39.4097' W, est. 1336–103; 4204 m deep, col. 24 April 2013, 3 specs.

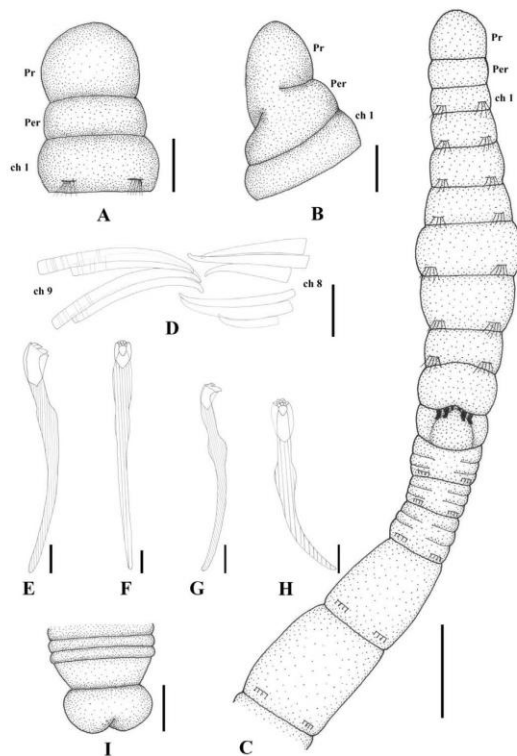
**Comparative material.** *Capitella perarmata*: (ZUEC POL 7635 and ZUEC POL 7636); Admiralty Bay, King George Island, Antarctica; 20 m; 01 January 1987.

**Description.** Size range of adults examined (complete individuals) 2.73–8.40 mm long (holotype 8.0 mm), 0.4–0.6 mm wide (holotype 0.5 mm) and 18–42 setigerous segments (holotype 42 setigerous segments); and juveniles 0.83–2.38 mm long, 0.19–0.21 mm wide and 12–17 setigerous segments. Specimens widest anteriorly, thoracic region only slightly wider than abdominal region, gradual narrowing posteriorly (Fig. 2B, C). Color in alcohol whitish.

Prostomium bluntly rounded, as wide as long (Figs. 1A, C and 2 B). Peristomium distinct forming a complete asetigerous segment, as wide as the prostomium, prominent dorsally and laterally (Figs. 1A, B, C and 2B, C); the oral opening is ventral (Figs. 1B and 2C); eyespot absent. Nuchal organs not visible neither under light microscopy nor under SEM.

Adults and juveniles specimens with setigers 1–4 similar in





**Fig. 1.** *Capitella iatapiuna* sp. nov. (A) Anterior end, dorsal view; (B) anterior end, lateral view; (C) incomplete male specimen, dorsal view; (D) genital spines; (E) thoracic hooded hook, lateral view; (F) thoracic hooded hook, frontal view; (G) abdominal hooded hook, lateral view; (H) abdominal hooded hook, frontal view; (I) pygidium, dorsal view. Pr: prostomium; Per: peristomium; Ch: chaetiger. Scale bars: A, B, I, 0.25 mm; C, 0.5 mm; D, 0.125 mm; E, F, G, H, 10  $\mu$ m.

shape (rectangular) and approximately as wide as peristomium; setigers 5–7 gradually increasing in width and length, with setiger 5 the widest; setigers 8 and 9 are similar in shape (quadrangular) and the narrowest; occasionally, all setigers with shallow intersegmental furrows, due to fixation; setigers 5–9 with ventral groove (Fig. 2C); only two adult individuals examined lacking genital spines. This species is apparently hermaphroditic because the specimens presented both genital spines and eggs in coelom.

Adult specimens presented capillaries in notopodia of setigers 1–7 and neuropodia of setigers 1–6; hooded hooks present in neuropodia of setigers 7–9; noto- and neurosetae arranged in a single row of 2–3 capillaries on setiger 1, 3–5 capillaries on setigers 2–7; and 3–7 hooded hooks on setigers 7–9. Juvenile specimens presented capillaries in noto- and neuropodia of setigers 1–3 and hooded hooks in noto- and neuropodia of setigers 4–9; noto- and neurosetae arranged in a single row of 1–2 capillaries and 1–3 hooded hooks. Capillary setae unilimbate, becoming narrow apically (Figs. 2E and 3E). Thoracic hooded hooks varying between 8.13 and 9.4  $\mu$ m in length; with details of dentition visible only using light microscopy at 1600 $\times$  or with SEM; main fang pointed, large, slightly curved, surmounted by 6 apical teeth arranged in two rows (3 basally and 3 in superior row); long shoulder slightly curved, short anterior shaft, inconspicuous node, slight constriction, long posterior shaft (Figs. 1E, F, 2F, G and 3A); hood visible in light microscopy, with main fang protruding through frontal

opening. Genital spine of setiger 8 visible externally, usually consisting of 2–3 pairs (Fig. 1C, D, 2B, D and 3C, D); these spines directed toward setiger 9; spines on setiger 9 imbedded, consisting of 2 pairs, usually not visible externally (Fig. 1C, D and 3C, D). Spines of setiger 8 slightly curved, narrower than those of setiger 9, with large base and tips sharply curved. Spines of setiger 9 larger than those of setiger 8, also curved and with sharply curved tips (Figs. 1D, 2D).

Abdominal setigers longer than wide, twice longer than thoracic ones (Fig. 1C), with 3–7 hooded hooks in notopodia and 4–10 in neuropodia, reduced to 2–3 hooks in posterior setigers and one hook in far posterior setigers in noto and neuropodia; abdominal hooded hooks shorter than thoracic ones (6.25–6.9  $\mu$ m) (Fig. 1G, H); main fang pointed, large, slightly curved, surmounted by 8–9 apical teeth arranged in three rows (3 basally, 3 in middle row and 2–3 in superior row); long shoulder slightly curved, short anterior shaft, developed node and constriction, long posterior shaft (Fig. 1G, H, 2I, J and 3B); hood visible in light microscopy, with main fang protruding through frontal opening (Fig. 3B). In far posterior setigers neuropodial hooded hooks emerge from parapodial ridges (Figs. 2H and 3G). Pygidium simple lobe, without anal cirri (Figs. 1I, 2H and 3F, G).

Thoracic segments staining uniformly, except for the setigers 5–7 that stain prominently; abdominal segments with uniform sparkles (Fig. 2K).

**Molecular identity.** The sequencing of the COI failed for all samples. A 16S fragment of 447 bp was obtained from two individuals, of the same haplotype; in consequence, the 16S intra-specific genetic distance was 0%. The 16S inter-specific genetic distance between *C. iatapiuna* and *C. teleta* (the only 16S sequence for *Capitella* available in GenBank) was of 20% (*p*-distance-K2P). The 16S sequences were deposited in GenBank under the accession numbers, KU160141 and KU160142.

**Etymology.** The species was named in tribute of the expedition name “Iatá-Piuna” that means “Navigating in Deep and Dark Waters” in the Tupi-Guarani, the first native Brazilian indigenous language.

**Type locality.** São Paulo Ridge-Southwest Atlantic (28° 31.1191' S 41° 39.4097'W).

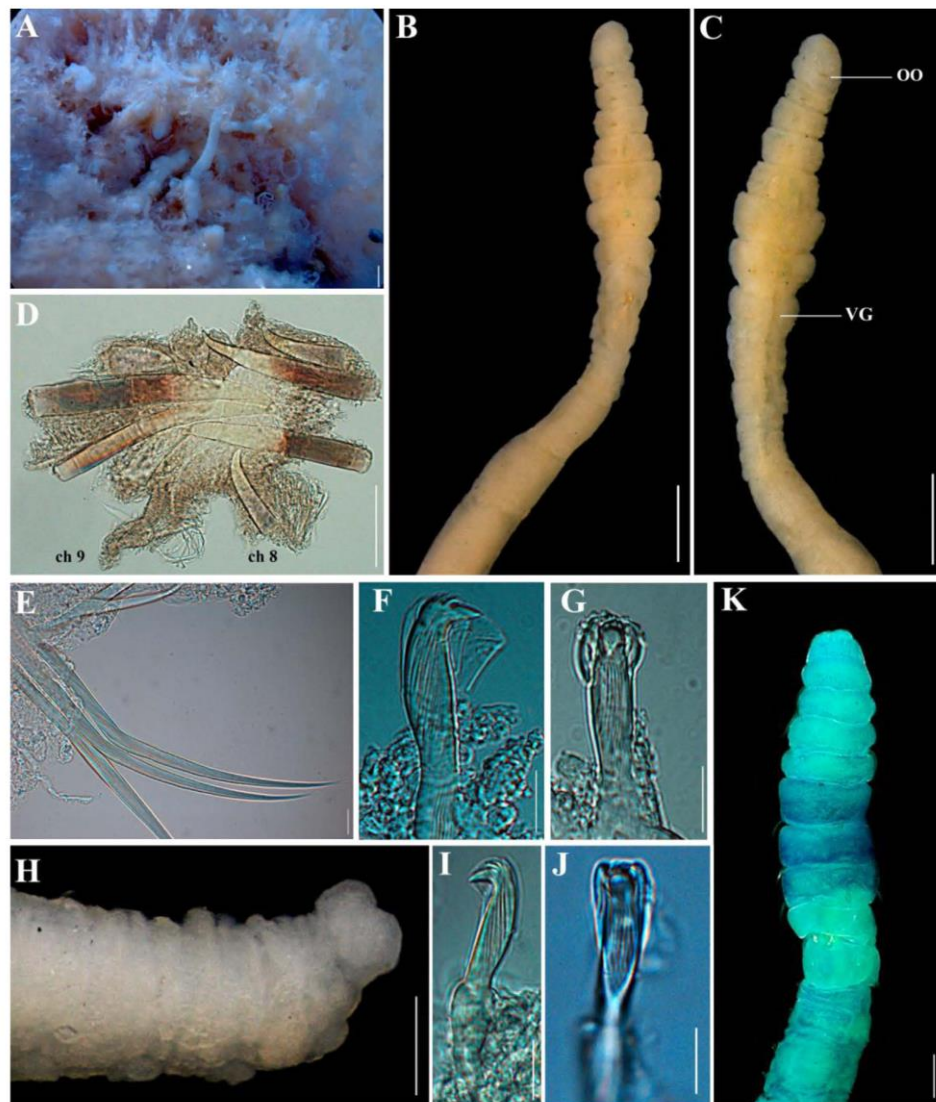
**Habitat.** The specimens of *C. iatapiuna* were found at 4204 m depth, in high abundance inside whalebones and in the sediments around the whale carcass.

## 4. Discussion

### 4.1. Biological and taxonomic remarks

*Capitella* species exhibit a great deal of diversity in life history and reproduction strategies, including gonochorism and hermaphroditism (Petraits, 1985). Laboratory experiments found that low densities in a population can promote the development of female gonads in males of *Capitella* resulting in simultaneous hermaphroditic animals (Holbrook and Grassle, 1984). This result may be caused by the increase of food availability or by the absence of inhibitory effects from animals in close proximity to animals with female gonads. Since *C. iatapiuna* occurs in high abundances in the studied whale-fall community, we do not believe that the absence of mates that is causing the hermaphroditism on this species, but the large amount food available. On this scenario, extra energy may be channeled into the development of female reproductive traits without the need to detract energy from other animal structures (Holbrook and Grassle, 1984).

The genus *Capitella* contains 18 valid species (Table 1) all having the first setigers and/or peristomium wider than the prostomium. On the other hand, *C. iatapiuna* has a peristomium and the



**Fig. 2.** *Capitella iatapiuna* sp. nov. (A) Individuals inside the whale bone matrix (B) anterior end of a male specimen, dorsal view; (C) anterior end of a male specimen, ventral view; (D) genital hooks; (E) Capillaries chaeta; (F) thoracic hooded hook, lateral view; (G) thoracic hooded hook, frontal view; (H) Pygidium, dorso-lateral view; (I) abdominal hooded hook, lateral view; (J) abdominal hooded hook, frontal view; (K) Methyl green staining. OO: oral opening. VG: ventral groove. Scale bars: A, 1 mm; B, C, 0.5 mm; D, E, 0.1 mm; F, G, I, J, 10 µm; K, 0.2 mm.

first two setigers with about same width of the prostomium. Setigers following the second increase in width until the fifth setiger and then decrease in the coming thoracic setigers. This remark is characteristic of the new species and it is present in juveniles and adults.

*C. iatapiuna* is similar to *Capitella caribaeorum* Warren and George (1986) in the morphology of the peristomium and in the setal formula. However, both species can be distinguished apart by the presence in *C. caribaeorum* of a roughly conical prostomium with a marked longitudinal ventral cleft; eyes in juveniles; similar thoracic and abdominal hooded hooks, with numerous teeth arranged in four rows, in comparison to different thoracic and

abdominal hooded hooks regarding the length of hooded hooks, number and arrangement of the teeth in *C. iatapiuna*.

*Capitella giardi* (Mesnil, 1897) has a setal formula alike to *C. iatapiuna*, nevertheless the former species differs from the latter in having the peristomium as an incomplete ring and similar thoracic and abdominal hooded hooks, with eight teeth in four rows (3 basally, mid one enlarged, 4 in middle row and 3–4 in superior row).

Although *Capitella aberranta* Hartman and Fauchald (1971) has also been found at abyssal depths (4862 m) presenting a dorsally conspicuous peristomium as a complete ring, this species is much smaller (1.9 mm length) than *C. iatapiuna* (8.4 mm length). It also



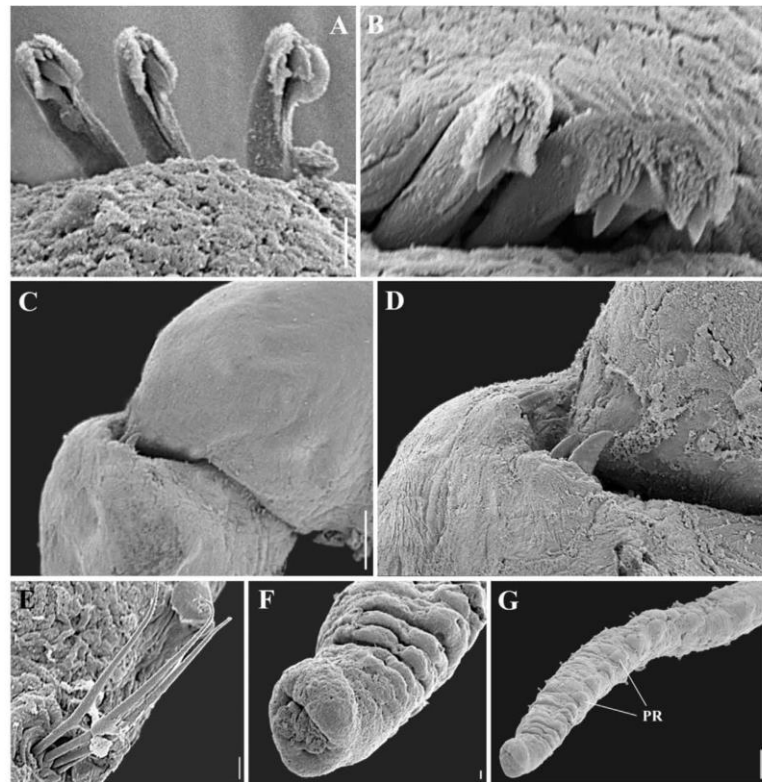


Fig. 3. *Capitella iatapiuna* sp. nov., SEM. (A) Thoracic hooded hook; (B) abdominal hooded hook; (C) setigers 8–9 and genital hooks; (D) genital hooks in detail; (E) Capillary chaetae; (F) pygidium and posterior end; (G) parapodial ridges (PR). Scale bars: A, 5 µm; B, 1 µm; C, G 100 µm; D, E, F, 10 µm.

has a conical prostomium and presents modified thoracic notochaeta, which are distally expanded and terminate in a slender projection.

Finally, *C. perarmata* was also registered living inside whale bones from Antarctic waters. However, this species presents a dorsally inconspicuous peristomium as an incomplete ring, neuropodia of setigers 8 and 9 mixed with hooded hooks and capillaries, hooded hooks with a single small tooth above the main fang and individuals reaching 80.0 mm in length.

Levels of 16S gene divergence (K2P distance=20%) between *C. iatapiuna* and *C. teleta* were in the same range of another Brazilian polychaete species *Timarete* spp. (18–26.4%) (Magalhães et al., 2014). Other polychaete species, such as *Paranaites* spp. (1.5%) (Nygren et al., 2009), *Marenzelleria* spp. (2.2–4.3%) (Bastrop et al., 1998) and *Myrianida* spp. (2.3%) (Nygren and Sundberg, 2003), were considered different despite having much lower genetic divergences (Bastrop et al., 1998; Nygren and Sundberg, 2003; Nygren et al., 2009). Therefore, the high genetic divergence and the several distinct taxonomic characters reported here indicate that *C. iatapiuna* can be considered a distinct species.

#### 4.2. Ecological comments

To the best of our knowledge, *C. iatapiuna* is the first *Capitella* described for deep-sea whale-fall communities. This genus has been reported in such communities in shallow waters (Taboada et al., 2013, 2015a,b) and only recently in deep-sea bone-

implanted experiments (Amon et al., 2015). The absence of this genus in deep-sea whale-fall communities has been considered a major taxonomic difference from shallow-water whale carcasses (Smith et al., 2014).

Most research in both shallow and deep-sea whale-fall communities has focused on the skeleton epifauna as well as on the sediment epi- and infauna. Methods such as video image analysis for megafauna identification have been widely used (Smith et al., 1989, 1998b, 2002, 2014; Bennett et al., 1994; Baco and Smith, 2003; Smith and Baco, 2003; Debenham et al., 2004; Goffredi et al., 2004; Dahlgren et al., 2006; Braby et al., 2007; Pavlyuk et al., 2009; Lundsten et al., 2010a,b; Glover et al., 2010). However, capitellids have been found whenever scientists carefully check the skeleton infaunal assemblages (Fujiwara et al., 2007; Taboada et al., 2013, 2015a,b; Amon et al., 2015). This suggests that *Capitella* may be a common inhabitant in whalebones and that their historical absence in whale falls is related to the lower effort spent to sort inner bone organisms.

One of the most striking aspects of our finding was the high abundance of *C. iatapiuna* inside bones in the deep Atlantic basin (Alfaro-Lucas et al., in preparation). Many *Capitella* species show high abundance in organic-rich areas (Blake et al., 2009) and it is known that they can be tolerant to anoxia and high sulfide conditions (Gamenick et al., 1998). Even though high sulfide levels are lethal for many organisms, some *Capitella* species could use this chemical compound as a cue for larval settlement (Cuomo, 1985). However, the role of sulfide as a settlement cue is doubtful since

**Table 1**Comparison among *Capitella* species. F: female; M: male; L: length; W: width; MGSP: Methyl green staining pattern; A: acicular chaeta; C: chaeta; H: hook; GH: genital hook; Mo: modify chaeta; Mi: mixed.

| Species  | Prostomium/<br>Peristomium                                   | Eyes         | Setal formula F/M   | Thoracic<br>hooks         | Abdominal<br>hooks        | Maximum<br>size (L × W,<br>mm <sup>2</sup> ) | MGSP   | Type locality                        | Habitat   | References   |
|--|--|--------------|---|---------------------------|---------------------------|--|--|--------------------------------------|---|--|
| <i>Capitella aberranta</i> Hartman and Fauchald (1971)   | Conical (dorsal depression)/Complete ring                    | Absent       | Noto: 5C/4Mo<br>Neuro: 5C/4H<br>Noto: 5C/4Mo<br>Neuro: 5C/4H            | –                         | –                         | 1.9 × 0.3                                    | –  | NW Atlantic Ocean, off New England   | Abyssal depth; 4862 m   | Hartman and Fauchald (1971) (Plate 11, Figs. G, H)   |
| <i>Capitella aciculata</i> (Hartman, 1959)               | Triangular (mid-dorsal depression)/Incomplete ring           | Absent       | Noto and neuro: 8C/1H<br>Noto: 2A/5C/2GH<br>Neuro: 8C/1H                | 5 teeth in 2 rows         | 5 teeth in 2 rows         | 35.0 × 1.8                                   | –  | St. Andrews Bay, Florida, California | –   | Hartman (1959) (Plate 1, Figs. 1–3)  |
| <i>Capitella capitata</i> (Fabricius, 1780)              | Short and rounded (mid-dorsal depression)/Incomplete ring    | Absent       | Noto: 7C/2H<br>Neuro: 7,8,9C/1–2H<br>Noto: 7C/2GH<br>Neuro: 7,8,9C/1–2H | Numerous teeth in 5 rows  | Numerous teeth in 5 rows  | 45.0 × 1.5                                   | Setigers 6–9 staining darkly   | West Greenland                       | Shallow depth; until 115 m; bottom with sand-mud and rocks and gravel | Blake (2009) (Figs. 2–5)   |
| <i>Capitella capitata floridana</i> Hartman (1959)       | Equitriangular/Incomplete ring                               | Absent       | Noto and neuro: 4C/5H<br>Noto: 4C/3H/2GH<br>Neuro: 4C/5H                | 4 teeth in a single row   | 4 teeth in a single row   | 6.2 × 0.56                                   | –  | St. Andrews Bay, Florida, California | In <i>Loligo</i> egg capsules   | Hartman (1959) (Plate 3, Figs. 4–6)  |
| <i>Capitella caribaeorum</i> Warren and George (1986)    | Conical (dorsal depression and ventral groove)/Complete ring | In juveniles | Noto: 7C/2H<br>Neuro: 6C/3H<br>Noto: 7C/2GH<br>Neuro: 6C/3H             | Numerous teeth in 4 rows  | Numerous teeth in 4 rows  | 20.0 × 0.7                                   | –  | Southern Florida                     | Intertidal; red mangrove  | Warren and George (1986) (Figs. 1–3)   |
| <i>Capitella dizonata</i> Johnson (1901)                 | Conical/Incomplete ring                                      | Absent       | Noto and neuro: 7C/2H<br>Noto: 7C/2GH<br>Neuro: 7C/2H                   | 4 teeth in a single row   | 4 teeth in a single row   | 36.0 × 1.5                                   | –  | Pacific Ocean, Washington            | Intertidal zone   | Johnson (1901) (Figs. 119–121)   |
| <i>Capitella giardi</i> (Mesnil (1897))                  | Conical/Incomplete ring                                      | Absent       | Noto and neuro: 6C/3H<br>Noto: 6C/1H/2GH<br>Neuro: 6C/3H                | 11–12 teeth in 4 rows     | 11–12 teeth in 4 rows     | 19.0 × 0.8                                   | Lateral setigers 7–8 intensely stained   | Atlantic Ocean, Cacuo, Angola        | –   | Warren (1976); Magalhães and Bailey-Brock (2012) (Figs. 1A–C, 2A–D and 3C–E)                     |
| <i>Capitella gracilis</i> (Verrill, 1880)                | Conical/–  |              | Noto and neuro: 6C/3H<br>Noto: 6C/1H/2GH<br>Neuro: 6C/3H                | –                         | –                         | 40.0 × 0.5                                   | –  | Atlantic Ocean, Connecticut          | Shallow depth; in mud   | Verrill (1880)   |
| <i>Capitella hermaphrodita</i> Boletzky and Dohle (1967) | Conical/Complete ring  | Present      | Noto and neuro: 4C/5H<br>Noto: 4C/3H/2GH<br>Neuro: 4C/5H                | 10 teeth in 2 rows        | 10 teeth in 2 rows        | 34.0 × –                                     | –  | France                               | In <i>Loligo</i> egg capsules   | Boletzky and Dohle (1967) (Fig. 1)   |
| <i>Capitella jonesi</i> (Hartman, 1959)                  | Equitriangular/Incomplete ring                               | Present      | Noto and neuro: 3C/6H<br>Noto: 3C/4H/2GH<br>Neuro: 3C/6H                | 3 teeth in a single row   | 3 teeth in a single row   | 15.0 × 1.0                                   | Setiger 6 slightly darker than others  | St. Andrews Bay, Florida, California | –   | Hartman (1959) (Plate 2, Figs. 1–3); Magalhães and Bailey-Brock (2012) (Figs. 4A–C, 5A–F and 6A) |
| <i>Capitella minima</i> Lan-gerhans (1881)               | Triangular/Complete ring                                     | Absent       | Noto: 4C/5H<br>Neuro: 3C/6H<br>Noto: 4C/3H/2GH<br>Neuro: 3C/6H          | 5–6 teeth in 2 rows       | 5–6 teeth in 2 rows       | 7.0 × 0.15                                   | Setigers 8–9 staining dark, often first two abdominal setigers intensely stained | Indian Ocean                         | Coarse sand; 70 m   | Green (2002) (Fig. 4A–F); Magalhães and Bailey-Brock (2012) (Figs. 6B, 7A–E, 8A–F)               |
| <i>Capitella ovincola</i> Hartman (1947)                 | Conical/Complete ring  | Absent       | Noto and neuro: 4C/3Mi/2H<br>Noto: 4C/3Mi/2GH<br>Neuro: 4C/3Mi/2H       | 4–5 teeth in a single row | 4–5 teeth in a single row | 60.0 × 1.5                                   | –  | Monterrey Bay, Florida, California   | In <i>Loligo</i> egg capsules; 30–98 m                                | Hartman, 1947 (Plate 44, Figs. 1–6); Blake (2000) (Fig. 4.4)                                     |
| <i>Capitella perarmata</i>                               | Rounded (dorsal  | Absent       | Noto: 7C/2H   | 1 small tooth             | 1 small tooth in          | 80.0 × 4.0                                   | –  | King George                          | Rocky shore to  | Gravier (1911)   |



| Gravier (1911)   | depression/In-complete ring                                   | Neuro: 7C/2Mi<br>Noto: 7C/2GH<br>Neuro: 7C/2Mi                        | in a single row                        | a single row                            | Island, South<br>Shetland Islands,<br>Antarctic | shallow water (10<br>m)   | (Plate VIII,<br>Figs. 88–93;<br>Plate IX,<br>Figs. 94–108)                  |
|--|---|---|--|---|---|---|---|
| <i>Capitella singularis</i><br>Fauvel (1932)               | Conical/Complete<br>ring                                      | Noto and neuro: 7C/2H<br>Noto: 7C/2GH<br>Neuro: 7C/2H                 | Numerous<br>(11–14) teeth<br>in 2 rows | Numerous (11–<br>14) teeth in<br>2 rows | India   | –   | Megahales and<br>Bailey-Brock<br>(2012) (Figs. 3A–<br>B, 9A–D and<br>10A–F) |
| <i>Capitella teleta</i> Blake<br>et al. (2009)             | Triangular (weak dor-<br>sal depression)/In-<br>complete ring | Noto and neuro: 7C/2H<br>Noto: 7C/2GH<br>Neuro: 7C/2H                 | 6 teeth in<br>2 rows                   | 6 teeth in<br>2 rows                    | Woods Hole, Mas-<br>sachusetts, USA             | Intertidal to shal-<br>low water  | Blake et al.<br>(2009) (Figs. 1–<br>3)                                      |
| <i>Capitella teres</i> Tread-<br>well (1939)               | –/–   | Noto and neuro: 8C/1H<br>Noto and neuro:<br>8C/1H                     | –                                      | –                                       | Port Aransas,<br>Texas, USA                     | –   | Treadwell (1939)  |
| <i>Capitella triparita</i><br>Hartman (1961)               | Conical/–   | Noto and neuro:<br>3C/4Mi/2GH<br>Noto: 3C/4Mi/2GH<br>Neuro: 3C/4Mi/2H | –                                      | –                                       | Pacific Ocean,<br>Southern<br>California        | In <i>Loigo</i> egg cap-<br>sules, fine mud,<br>coarse sand, and<br>pebbles; 46–119 m | Hartman (1961)<br>and Warren<br>(1976)                                      |
| <i>Capitella minima tu-<br/>keensis</i> Thomasin<br>(1970) | Conical/Complete<br>ring                                      | Noto and neuro: 3C/6H<br>Noto: 3C/4H/2GH<br>Neuro: 3C/6H              | 7–9 teeth in<br>3 rows                 | 7–9 teeth in<br>3 rows                  | Indian Ocean, Tu-<br>lear, Madagascar           | In corals   | Thomasin (1970)<br>(Fig. 2)   |
| <i>Capitella iatapiuna</i> sp.<br>nov.                     | Rounded/Complete<br>ring                                      | Noto: 7C/2H<br>Noto: 6C/3H<br>Noto: 7C/2GH<br>Neuro: 6C/3H            | 5–6 teeth in<br>2 rows                 | 8–9 teeth in<br>3 rows                  | Southwest Atlantic                              | In whale bones;<br>4204 m   | This study  |

the larval response to sulfide occurs with a delay of many hours (Dubilier, 1988). The direct response to sulfide is unclear, but it has been observed that *C. teleta*, a sibling species of *C. capitata*, had higher survival and growth rates in sulfide-rich environments, as it selective feeds on chemosynthetic bacteria (Tsutsumi et al., 2001). The supply of labile organic matter from chemosynthetic production may also be an important factor controlling the distribution and population dynamics of many *Capitella* species (Tsutsumi et al., 2001), possibly including *C. iatapiuna*.

The bone inner parts are thought to be reducing anaerobic environments (Deming et al., 1997; Treude et al., 2009; Huusgaard et al., 2012). Bone degradation processes occur progressively from the outside towards the center and are mediated mainly by anaerobic microbial activities and limited by water compounds diffusion, which act as electron donors (Deming et al., 1997; Goffredi et al., 2008; Treude et al., 2009; Smith et al., 2015). Some species such as *Osedax* may accelerate this process since their feeding activities strongly erode bones and facilitate inner-bone colonization by microbes (Higgs et al., 2011). We do not believe that *C. iatapiuna* actively erodes bones like *Osedax* do using a proton pump acidifying mechanism (Tresguerres et al., 2013). On the contrary, *C. iatapiuna* was observed inhabiting the available space of the bone trabecular system without causing physical degradation (Fig. 2A) (Alfaro-Lucas et al., in preparation).

*Capitella* behavior normally enhances the bacterial growth in organically enriched sediments, which can contribute to an increase in its own food resources (Kunihiro et al., 2011). This association of bacteria with *Capitella* promotes a rapid decomposition of organic matter in organically enriched sediments (Kunihiro et al., 2008, 2011). Thus, if *C. iatapiuna* exhibits a similar behavior inside bones, it probably accelerates bone degradation influencing in the longevity of the whale-fall community. The activity of *C. iatapiuna* may facilitate the entrance of water/sediment compounds to bone inner parts enhancing the degradation through the stimulation of other metabolisms and/or microbial growth (Alfaro-Lucas et al., in preparation).

For some widespread polychaete genera, deep-sea whale-fall communities are important habitats for their dispersion and evolution in the deep-sea (Wiklund et al., 2009). *Capitella* species are very common in organic-rich habitats including vents and seeps (e.g., Pearson and Rosenberg (1978), Davis and Spies (1980), Gamenick et al. (1998) and Levin et al. (2009)). We believe that chemosynthetic environments may have played an important role for the colonization and dispersal of the genus *Capitella* in the deep-ocean. We have already found other *Capitella* species yet to be identified/described in both a shallow and deep-water whale-fall experiments in the Southwest Atlantic Ocean as dominant species. Therefore, we suggest these patchily distributed habitats may be important for the dispersion and evolution of the genus in the deep Southwest Atlantic Ocean.

## 5. Conclusion

The genus *Capitella* has only recently been reported in deep-sea whale-fall communities and *C. iatapiuna* is the first described species for such communities. Supply of labile organic matter from chemosynthetic production and the adaptation for environments with high concentration of sulfide may be important factors controlling the distribution and population dynamics of this species. Chemosynthetic environments may have played an important role for the colonization and dispersal of the genus *Capitella* in the deep-ocean. The occurrence of hermaphroditism in *C. iatapiuna* has been linked to the opportunistic life history of the group. The inter-specific genetic distance was very high warranting the specific status for *C. iatapiuna*.



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## CAPÍTULO 2

### **Demystifying the *Capitella capitata* complex (Annelida, Capitellidae) diversity by morphological and molecular data along the Brazilian coast**

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## RESEARCH ARTICLE

Demystifying the *Capitella capitata* complex (Annelida, Capitellidae) diversity by morphological and molecular data along the Brazilian coastCamila F. Silva<sup>1\*</sup>, Victor C. Seixas<sup>2\*</sup>, Rômulo Barroso<sup>3\*</sup>, Maikon Di Domenico<sup>4‡</sup>, Antonia C. Z. Amaral<sup>1‡</sup>, Paulo C. Paiva<sup>2‡</sup>

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## Abstract

The sibling species of *Capitella capitata* are globally known for their tolerance to disturbed habitats and the *C. capitata* complex is often used as an ecological indicator. A recent re-description proposed that *C. capitata*, originally described in Greenland is restricted to the Arctic and Subarctic regions. Given their ecological relevance, we conducted a morphological and molecular analyses based on mtDNA sequences to investigate the diversity and distribution of the *C. capitata* complex along the Brazilian coast. Our morphological and molecular data were congruent and revealed the existence of four new species distinct from *C. capitata*, collected from the type locality. This study is the first characterization of the biodiversity and distribution of *Capitella* species made along the Brazilian coast and yielded a set of morphological characters corroborated by the mtDNA sequences for species identification. Our results increase the biodiversity of the genus along the Brazilian coast by describing four new species (*Capitella aracaensis* sp. n., *Capitella biota* sp. n., *Capitella neoaciculata* sp. n. and *Capitella nonatoi* sp. n.). One species was collected from only one sampling site, while the others are distributed along the coast.

## Introduction

The genus *Capitella* Blainville 1828 [1] (Capitellidae, Annelida) comprises polychaetes characterized by nine thoracic chaetigers with a species variation associated with the number of chaetigers bearing capillary chaetae and hooded hooks, genital spines present in chaetigers 8–9 of males and hermaphrodites, and the pygidium lacking appendages. There are 19 nominal species of *Capitella* described in a variety of environments (e.g., intertidal zone to abyssal depths) and habitats (e.g., soft-bottom, egg capsules, whale-bones) worldwide [2–5].

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General attributes traditionally used to characterize capitellid species include overall size, shape and relative size of the prostomium and peristomium, formation of the peristomium as a complete or an incomplete ring, the number and distribution of capillary chaetae and hooded hooks along the thorax, the morphology of the genital spines, the number, size and structure of the hooded hooks and the shape of the pygidium [2–6]. However, distinguishing *Capitella* species is difficult and includes multiple complexes of cryptic species, which morphology alone has failed to define since the morphological features and life history traits can overlap.

The genus *Capitella* was the first marine invertebrate genus identified as a complex of cryptic species through molecular markers (allozyme) in the pioneer work of Grassle & Grassle [7], in which six different species, previously considered as *Capitella capitata*, were discovered along a short stretch of the USA Atlantic coast. Subsequent studies also identified cryptic species of *Capitella* through differences in their reproductive modes [8–9], gamete and larvae ultrastructure [10–11], developmental rates, dispersal patterns [12–14], adult body sizes [15] and physiological characteristics [16–17]. These criteria were used to define at least twelve cryptic species that were previously identified in laboratory cultures [3].

*Capitella capitata* [18] was originally described in Greenland, but the reports of its occurrence were later expanded to all oceans [3, 19–21]. The poor taxonomic understanding of the complex of cryptic species has led to broad distributions of these taxa, also known as ‘the cosmopolitan syndrome’ [22–23]. However, careful morphological revision of specimens and the advent of molecular techniques have increased the local diversity of several polychaete species and diminished the geographical distribution of species previously considered to be cosmopolitan [24–29].

After his re-description of *C. capitata* from the type locality, Blake [3] proposed this species is restricted to the Arctic and Subarctic regions. Specimens of *C. capitata* that were recorded along the Brazilian coast [30–31] were therefore apparently misidentified. Moreover, the records of *C. capitata* from diverse benthic habitats and depths throughout the world indicate that a number of sibling and undescribed species are being overlooked taxonomically and ecologically.

*Capitella capitata* is referred to as an important ecological indicator, due to its high densities in polluted ecosystems [32–33] and as a model organism in many ecotoxicological studies [17, 34–35]. However, ecological studies are likely referring to an unknown number of species with different local and regional distributional patterns, toxicity tolerance [17], and reproductive strategies [34]. Given the ecological importance of this group the correct delimitation of species is essential. The aims of the present study were to investigate the diversity and distribution of the Brazilian populations of the *C. capitata* complex and characterize them morphologically and molecularly. Morphological characters and mtDNA sequences (COI and 16S) of specimens from 13 sites along the Brazilian coast were analyzed. Sequences from the type locality and public datasets were also included. The results will be useful in order to correctly identify the species in further distinct studies.

## Material and methods

### Study areas and sampling

Samples were collected from the intertidal zone and shallow waters (up to 0.5 m deep) at 13 sites along the Brazilian coast in six different states (Fig 1). Samples were also collected at one site in Greenland (02-Aug-2013; 69.25°S 54.10°W). Collected sediments were washed in the field on a 500 µm and 300 µm mesh sieve and any *Capitella* worms retained in the residue were fixed and preserved in 92% ethanol.

the maximum likelihood (ML) algorithm and Bayesian inference (BI). Three datasets were considered, COI, 16S and concatenated (COI+16S). The ML trees were estimated in RAXML 8.2 [43] using the substitution models GTR+G for all datasets. The best ML tree was obtained from 20 initial independent trees, and the statistical support was obtained with a rapid bootstrap function (-f a) using 1000 replicates. The BI was conducted in MrBayes 3.2 [44] under the HKY+I+G model for COI and 16S, and HKY+G for the concatenated dataset, as selected in the jModelTest 2 [45] using Bayesian information criterion (BIC). Tree parameters were sampled every 1000 generations, for a total of  $10^7$  samples. Two independent runs and 4 chains were implemented. The final results were checked according to the standard deviations of split frequencies ( $<0.01$ ). The effective sampling size (ESS $>200$ ) was assessed via Tracer 1.5 [46]. The ML and BI analyses were conducted in CIPRES Science Gateway [47]. Species from two other Capitellidae genera, *Notomastus* (16S and COI) and *Heteromastus* (COI), were considered as outgroups and were used to root the trees. The concatenated dataset used only *Notomastus* as an outgroup as no 16S sequences are available for *Heteromastus*. Intra- and interspecific pair-wise genetic distances were estimated under the *p*-distance and Kimura 2 parameters models with MEGA 6.1 [48]. GenBank sequences of *C. capitata* from other localities were taken to compare with our sequences. Data collection of specimens, museum codes and GenBank ([www.ncbi.nlm.nih.gov/genbank](http://www.ncbi.nlm.nih.gov/genbank)) accession numbers are detailed in Table 1.

## Results

### Morphological analysis

Examination of 4,423 specimens from different localities along the Brazilian coast allowed us to classify them into four species within *Capitella*: 33 *C. aracaensis* sp. n., 149 *C. biota* sp. n., 734 *C. neoaciculata* sp. n., and 3,507 *C. nonatoi* sp. n. These species were identified mainly by the overall size, shape and size of the prostomium and peristomium, formation of the peristomium as a complete or an incomplete ring, the number and distribution of capillary chaetae and hooded hooks along the thoracic chaetigers, the details of the genital spines, the number, size and structure of the hooded hooks and the shape of the pygidium. The differences of these characteristics among the *Capitella* species were already summarized in a table by Silva et al. [5]. Here, we provide the description of the species and a key to the new species among all valid *Capitella* species.

### Taxonomic account

Family Capitellidae Grube, 1862 [49]

Genus *Capitella* Blainville, 1828 [1]

Type species. *Capitella capitata* (Fabricius, 1780) as *Lumbricus capitatus*. [18] Redescribed by Blake, 2009 [3].

Type locality. West Greenland.

**Diagnosis (emended after Magalhães & Bailey-Brock, 2012 [4]).** Prostomium conical to bluntly rounded, sometimes dorsoventrally flattened, with a dorsal groove present or absent, with nuchal organs as paired slits at border between prostomium and peristomium; eyespots present or absent. Peristomium forming a complete or an incomplete chaetous ring. Thorax with 10 segments including an achaetous peristomium and nine chaetigers. Capillary chaetae in both rami of chaetigers 1–3, 1–4, 1–6, or 1–7 or capillaries and hooks in various combinations in both rami, and chaetigers 8–9 with hooded hooks, mixed capillaries and hooks, or all capillaries; arrangements sometimes size dependent. Genital hooks present in chaetigers 8–9 of males and hermaphrodites; females usually with enlarged lateral genital pores between chaetigers 7–8 or 8–9. Capillaries unilimbate, hooded hooks with multiple rows of denticles above

The nomenclature used for chaetal morphology follows that usually used for Capitellidae species and some suggested by Green [2]. The chaetal characters used here are:

- main fang: format and angle with the hook shaft;
- teeth: number of teeth and rows that are arranged above the main fang;
- shoulder: development of the curvature of the hook;
- hood: size, format, size of the opening and texture;
- anterior shaft: length between shoulder and node;
- node: presence or absence and shape;
- posterior shaft: length between node and the end of the hook.

The material was deposited at the Museu de Zoologia, Universidade Estadual de Campinas (ZUEC), São Paulo, Brazil and the Museu Nacional do Rio de Janeiro (MNRJ), Rio de Janeiro, Brazil.

### Nomenclatural acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix "<http://zoobank.org/>". The LSID for this publication is: urn:lsid:zoobank.org:pub:7C72C6E6-F616-4A60-87D7-C2239F03AB14. The electronic edition of this work was published in a journal with an ISSN, and has been archived and is available from the following digital repositories: PubMed Central, LOCKSS.

### Molecular analysis

**DNA extraction, amplification and sequencing.** Genomic DNA was extracted with a [DNeasy Blood & Tissue Kit \(QIAGEN\)](#) and in some cases according to the protocol in Floyd et al. [36]. Fragments of the mitochondrial gene, cytochrome oxidase subunit I (COI), were amplified using universal primers LCO1490 and HCO2198 [37] or degenerated primers degLCO1490 and degHCO2198 [38]. For the 16S ribosomal DNA (16S) the universal primers 16Sar-L and 16Sbr-H [39] were used. PCR reactions for both loci consisted of PuReTaq Ready-To-Go™ PCR Beads (GE Healthcare), 1.5 µL of each primer (0.6 mM), 2 µL of DNA and 20 µL of water. The thermal cycling conditions for COI were one cycle of 94°C for 3 min, 5 cycles of 94°C for 40 s, 47°C for 40 s and 72°C for 1 min, 32 cycles of 94°C for 40 s, 52°C for 40 s and 72°C for 1 min, followed by a final extension step of 72°C for 5 min. For the 16S amplification, the reaction consisted of one cycle of 94°C for 3 min, 5 cycles of 94°C for 30 s, 42°C for 40 s and 72°C for 90 s, 32 cycles of 94°C for 30 s, 46°C for 40 s and 72°C for 90 s, followed by a final extension step of 72°C for 7 min. The resulting PCR products were purified and sequenced by Macrogen, Inc.

**Data analysis.** Electropherograms were edited with Sequencher 4.1 (Gene Codes Corporation), and sequences were aligned with MAFFT 7.0 [40] using the G-INS-I strategy for COI and Q-INS-I for 16S. COI sequences were checked for translation using the invertebrate mitochondrial genetic code. As saturation was not detected in the saturation test [41], all codon positions were used, as implemented in DAMBE5 [42]. The phylogenetic analysis was performed using



the maximum likelihood (ML) algorithm and Bayesian inference (BI). Three datasets were considered, COI, 16S and concatenated (COI+16S). The ML trees were estimated in RAXML 8.2 [43] using the substitution models GTR+G for all datasets. The best ML tree was obtained from 20 initial independent trees, and the statistical support was obtained with a rapid bootstrap function (-f a) using 1000 replicates. The BI was conducted in MrBayes 3.2 [44] under the HKY+I+G model for COI and 16S, and HKY+G for the concatenated dataset, as selected in the jModelTest 2 [45] using Bayesian information criterion (BIC). Tree parameters were sampled every 1000 generations, for a total of  $10^7$  samples. Two independent runs and 4 chains were implemented. The final results were checked according to the standard deviations of split frequencies ( $<0.01$ ). The effective sampling size (ESS $>200$ ) was assessed via Tracer 1.5 [46]. The ML and BI analyses were conducted in CIPRES Science Gateway [47]. Species from two other Capitellidae genera, *Notomastus* (16S and COI) and *Heteromastus* (COI), were considered as outgroups and were used to root the trees. The concatenated dataset used only *Notomastus* as an outgroup as no 16S sequences are available for *Heteromastus*. Intra- and interspecific pair-wise genetic distances were estimated under the *p*-distance and Kimura 2 parameters models with MEGA 6.1 [48]. GenBank sequences of *C. capitata* from other localities were taken to compare with our sequences. Data collection of specimens, museum codes and GenBank ([www.ncbi.nlm.nih.gov/genbank](http://www.ncbi.nlm.nih.gov/genbank)) accession numbers are detailed in Table 1.

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**Table 1. Collection information for specimens used in morphological and molecular analyses and GenBank accession numbers.**

| Species                               | Sample site                                 | Morphology |   | Molecular |  |                      |
|---------------------------------------|---|------------|---|-----------|--|----------------------|
|                                       |   | SPEC. #    |   | COL       | GenBank ID COL                         | 16S GenBank ID 16S   |
| <i>Capitella neoaciculata</i> sp. n.  | Maracalpe Mangrove, PE (8.31° S 35° W)      |            | .   | 3         | KX121855-KX121857                      | .                    |
|                                       | Guarapina Lagoon, RJ (22.57° S 42.42° W)    |            | .   | 3         | KX121828-KX121830                      | 3 KX121957-KX121959  |
|                                       | Maricá Lagoon, RJ (22.55° S 42.49° W)       |            | .   | 5         | KX121831-KX121835                      | 5 KX121960-KX121964  |
|                                       | Itaipú Lagoon, RJ (22.57° S 43.2° W)        | 45         | ZUEC POL: 17648                                     | 5         | KX121836-KX121840                      | 5 KX121965-KX121969  |
|                                       | Piratinga Lagoon, RJ (22.57° S 43.5° W)     | 34         | ZUEC POL: 17647                                     | 5         | KX121841-KX121845                      | 5 KX121970-KX121974  |
|                                       | Araçá Bay, SP (23.48° S 45.24° W)           | 655        | ZUEC POL: 16785–16825 / 17373–17436, MNRJP: 1429    | 9         | KX121846-KX121854                      | 14 KX121975-KX121988 |
| <i>Capitella aracaensis</i> sp. n.    | Araçá Bay, SP (23.48° S 45.24° W)           | 33         | ZUEC POL: 16779 / 17437–17458, MNRJP: 994           | 3         | KX121876-KX121878                      | 4 KX122007-KX122010  |
| <i>Capitella biota</i> sp. n.         | Todos os Santos Bay, BA (12.47° S 38.37° S) |            | .   | 10        | KX121866-KX121875                      | 9 KX121988-KX122005  |
|                                       | Araçá Bay, SP (23.48° S 45.24° W)           | 152        | ZUEC POL: 16726–16729 / 16731–16778, MNRJP: 997     | 8         | KX121858-KX121865                      | 9 KX121989-KX121997  |
| <i>Capitella nonatoi</i> sp. n.       | Carijó Bay, PA (0.49° S 46.36° W)           | 11         | ZUEC POL: 17852                                     | .         | .                                      | 3 KX122011-KX122013  |
|                                       | Maracalpe Mangrove, PE (8.31° S 35° W)      |            | .   | 1         | KX121827                               | 1 KX121956           |
|                                       | Todos os Santos Bay, BA (12.47° S 38.37° S) |            | .   | 9         | KX121818-KX121826                      | 11 KX121945-KX121955 |
|                                       | Aranua Lagoon, RJ (22.53° S 42.23° W)       |            | .   | 5         | KX121773-KX121777                      | 5 KX121880-KX121884  |
|                                       | Saquarema Lagoon, RJ (22.55° S 42.33° W)    |            | .   | 5         | KX121778-KX121782                      | 5 KX121885-KX121889  |
|                                       | Jaconé Lagoon, RJ (22.56° S 42.39° W)       |            | .   | 5         | KX121783-KX121787                      | 5 KX121890-KX121894  |
|                                       | Guarapina Lagoon, RJ (22.57° S 42.42° W)    |            | .   | 5         | KX121802-KX121806                      | 5 KX121895-KX121899  |
|                                       | Itaipú Lagoon, RJ (22.57° S 43.2° W)        | 15         | ZUEC POL: 17650                                     | 5         | KX121788-KX121792                      | 5 KX121900-KX121904  |
|                                       | Piratinga Lagoon, RJ (22.57° S 43.5° W)     |            | .   | 4         | KX121793-KX121796                      | 5 KX121905-KX121909  |
|                                       | Guanabara Bay, RJ (22.5° S 43.13° W)        |            | .   | 5         | KX121797-KX121801                      | 5 KX121910-KX121914  |
|                                       | Araçá Bay, SP (23.48° S 45.24° W)           | 3479       | ZUEC POL: 17459–17473 / 17581–17643, MNRJP: 995–996 | 7         | KX121811-KX121817                      | 21 KX121915-KX121935 |
|                                       | Paranaguá Bay, PR (25.30° S 48.29° W)       | 2          | ZUEC POL: 17651                                     | 4         | KX121807-KX121810                      | 9 KX121936-KX121944  |
|                                       | Greenland (69.25° N 54.10° W)               |            | .   | 1         | KX121879                               | 1 KX122014           |
|                                       | Indo-Pacific                                |            | .   | 4         | JX676137, JX676150, JX676171, JX676179 | .                    |
|                                       | Hudson Bay, Canada                          |            | .   | 6         | HQ023468-HQ023473, GU672407            | .                    |
| <i>Capitella</i> cf. <i>Capitata</i>  | Gulf of Mexico, Galveston, USA              |            | .   | 2         | KX961404, KX961414                     | .                    |
|                                       | Gulf of Mexico, Florida, USA                |            | .   | 2         | KX961432, KX961427                     | .                    |
| <i>Capitella</i> cf. <i>Aciculata</i> | Gulf of Mexico, Galveston, USA              |            | .   | 2         | KX961408, KX961411                     | .                    |
|                                       | Gulf of Mexico, Florida, USA                |            | .   | 2         | KX961424, KX961433                     | .                    |
| <i>Capitella teleta</i>               | Miyagi, Japan                               |            | .   | 3         | LC120627, LC120631, LC120638           | 1 JF509722           |
| <i>Capitella</i> aff. <i>teleta</i>   | Miyagi, Japan                               |            | .   | 3         | LC120644, LC120646, LC120650           | .                    |
| <i>Capitella</i> sp.                  | Hokkaido, Japan                             |            | .   | 1         | LC120652                               | .                    |

(Continued)

Table 1. (Continued)

| Species                        | Sample site | Morphology |  | Molecular |                   |     |                |
|--------------------------------|-------------|------------|--|-----------|-------------------|-----|----------------|
|                                |             | SPEC. #    |  | COI       | GenBank ID COI    | 16S | GenBank ID 16S |
| <i>Notomastus profundus</i>    |             |            |  | 1         | KR916897          | .   | .              |
| <i>Notomastus</i> sp.          |             |            |  | .         | .                 | 1   | KF511858       |
| <i>Notomastus hampodus</i>     |             |            |  | .         | .                 | 1   | HM746714       |
| <i>Heteromastus filiformis</i> |             |            |  | 2         | KR916852-KR916853 |     |                |

<sup>a</sup> PE, Pernambuco<sup>b</sup> RJ, Rio de Janeiro<sup>c</sup> SP, São Paulo<sup>d</sup> BA, Bahia<sup>e</sup> PA, Pará<sup>f</sup> PR, Paraná.<sup>g</sup> SPEC.<sup>h</sup> COI, cytochrome oxidase subunit 1<sup>i</sup> 16S, ribosomal DNA<sup>j</sup> ZUEC POL, Museu de Zoologia da Universidade Estadual de Campinas<sup>k</sup> MNRJ, Museu Nacional do Rio de Janeiro.

# specimen number

<https://doi.org/10.1371/journal.pone.0177760.t001>

the main fang. Abdominal segments with hooded hooks in both rami, without capillaries. Branchiae present or absent, and pygidium without appendages.

### Key to all valid species of *Capitella*

#### 1a. Capillary chaetae on chaetigers 1 – 3: 2

##### 1b. Capillary chaetae on chaetigers 1 – 4: 3

##### 1c. Capillary chaetae on chaetigers 1 – 5: 4

##### 1d. Capillary chaetae on chaetigers 1 – 6: 5

##### 1e. Capillary chaetae on chaetigers 1 – 7: 6

##### 1f. Capillary chaetae on chaetigers 1 – 8: 7

2a. Mixed chaetae and hooded hooks in noto- and neuropodia of chaetigers 4 – 7; hooded hooks on chaetigers 8 and 9; eyespots present; *C. capitata tripartita* Hartman 1961 [50]

2b. Hooded hooks on chaetigers 4 – 9; prostomium equitriangular; peristomium forming an incomplete achaetous ring; eyespots present; genital spines present; hooded hooks with three teeth above main fang in a single row: *C. jonesi* (Hartman 1959) [51]

2c. Hooded hooks on chaetigers 4 – 9; prostomium conical; peristomium forming a complete achaetous ring; eyespots absent; genital spines present; hooded hooks with 7 – 9 teeth above main fang arranged in three rows: *C. minima tulearensis* (Thomasin 1970) [52]

2d. Hooded hooks on chaetigers 4 – 9; prostomium conical; peristomium forming a complete achaetous ring; eyespots absent; genital spines present; thoracic hooded hooks with one apical tooth and abdominal hooks with two teeth above main fang, one above the other: *C. biota* sp. n.

3a. Mixed chaetae and hooded hooks in noto- and neuropodia of chaetigers 5 – 7; hooded hooks on chaetigers 8 and 9; prostomium conical; peristomium forming a complete achaetous ring; eyespots absent: *C. ovincola* Hartman 1947 [20]

**3b.** Hooded hooks on chaetigers 5–9; prostomium equitriangular; peristomium forming an incomplete achaetous ring; eyespots absent; hooded hooks with four teeth above main fang in a single row: *C. capitata floridana* Hartman 1959 [51]

**3c.** Hooded hooks on chaetigers 5–9; prostomium triangular; peristomium forming a complete achaetous ring; eyespots absent; hooded hooks with 5–6 teeth above main fang arranged in two rows: *C. minima* Langerhans 1881 [53]

**3d.** Hooded hooks on chaetigers 5–9; prostomium conical; peristomium forming a complete achaetous ring; eyespots present; hooded hooks with 10 teeth above main fang arranged in two rows: *C. hermaphrodita* Boletzky & Dohle 1967 [54]

**4a.** Modified chaetae in noto- and neuropodia of chaetigers 6–9; prostomium conical; peristomium forming a complete achaetous ring; eyespots absent: *C. aberrantia* Hartman & Fauchald 1971 [55]

**5a.** Hooded hooks on chaetigers 7–9; prostomium conical: *C. gracilis* (Verrill 1880) [56]

**5b.** Hooded hooks on chaetigers 7–9; prostomium conical; peristomium forming an incomplete achaetous ring; eyespots absent; hooded hooks with 10–12 teeth above main fang arranged in four rows: *C. giardi* (Mesnil 1897) [57]

**6a.** Hooded hooks in notopodia and mixed chaetae and hooded hooks in neuropodia of chaetigers 8 and 9; prostomium conical; peristomium forming an incomplete achaetous ring; eyespots absent; hooded hooks with one small tooth above main fang: *C. perarmata* (Gravier 1911) [58]

**6b.** Hooded hooks on chaetigers 8 and 9; prostomium short and conical, flattened dorso-ventrally; peristomium forming a complete achaetous ring; eyespots absent; thoracic chaetigers rugose; abdominal chaetigers with dark brown pigmented dorsum; hooded hooks with two rows of teeth above main fang: *C. amboensis* Pamunkgas 2017 [59]

**6c.** Hooded hooks on chaetigers 8 and 9; prostomium conical; peristomium forming a complete achaetous ring; eyespots absent; thoracic and abdominal hooded hooks with two teeth above main fang, one above the other: *C. aracaensis* sp. n.

**6d.** Hooded hooks on chaetigers 8 and 9; prostomium short and rounded with a mid-ventral depression; peristomium forming an incomplete achaetous ring; eyespots absent; hooded hooks with numerous teeth above main fang arranged in five rows: *C. capitata* (Fabricius 1780) [18]

**6e.** Hooded hooks on chaetigers 8 and 9; prostomium conical with a dorsal depression and a ventral groove; peristomium forming a complete achaetous ring; eyespots present in juveniles; hooded hooks with numerous teeth above main fang arranged in four rows: *C. caribacorum* Warren & George 1986 [60]

**6f.** Hooded hooks on chaetigers 8 and 9; prostomium conical; peristomium forming an incomplete achaetous ring; eyespots absent; hooded hooks with four teeth above main fang in a single row: *C. dizonata* Johnson 1901 [61]

**6g.** Hooded hooks on chaetigers 8 and 9; prostomium rounded; peristomium forming a complete achaetous ring; eyespots absent; thoracic hooded hooks with 5–6 teeth above main fang arranged in two rows; abdominal hooded hooks with 8–9 teeth above main fang arranged in three rows: *C. iatapiuna* Silva et al. 2016 [5]

**6h.** Hooded hooks on chaetigers 8 and 9; prostomium rounded with a dorsal smooth depression and ventral groove; peristomium forming an incomplete achaetous ring; eyespots present; thoracic hooded hooks with six teeth above main fang arranged in two rows; abdominal hooded hooks with three teeth above main fang arranged in two rows: *C. nonatoi* sp. n.

**6i.** Hooded hooks on chaetigers 8 and 9; prostomium conical; peristomium forming a complete achaetous ring; eyespots absent; hooded hooks with 11–14 teeth above main fang arranged in two rows; branchiae present: *C. singularis* (Fauvel 1932) [62]

6j. Hooded hooks on chaetigers 8 and 9; prostomium triangular; peristomium forming an incomplete achaetous ring; eyespots present; hooded hooks with six teeth above main fang arranged in two rows: *C. teleta* Blake et al. 2009 [6].

7a. Hooded hooks on chaetiger 9: *C. teres* (Treadwell 1939) [63].

7b. Acicular spines in noto- and neuropodia of chaetigers 1 and 2 of males and generally in notopodia of chaetiger 1 of females; prostomium triangular with smooth dorsal depression and ventral groove; peristomium forming an incomplete achaetous ring; eyespots absent; thoracic and abdominal hooded hooks with 5 teeth above main fang arranged in two rows: *C. aciculata* (Hartman 1959) [51].

7c. Acicular spines in noto- and neuropodia of chaetigers 1 and 2 of males and capillary chaetae on females; prostomium triangular with smooth dorsal depression and ventral groove; peristomium forming an incomplete achaetous ring; eyespots absent; thoracic and abdominal hooded hooks with 6 teeth above main fang arranged in two rows, pygidium large, heart-shaped: *C. neoaciculata* sp. n.

***Capitella aracaensis* sp. n.** Silva & Amaral Figs 2–4. urn:lsid:zoobank.org:act:1B37F3B-F-EB6C-4E57-8C9F-677B8AA92F43.

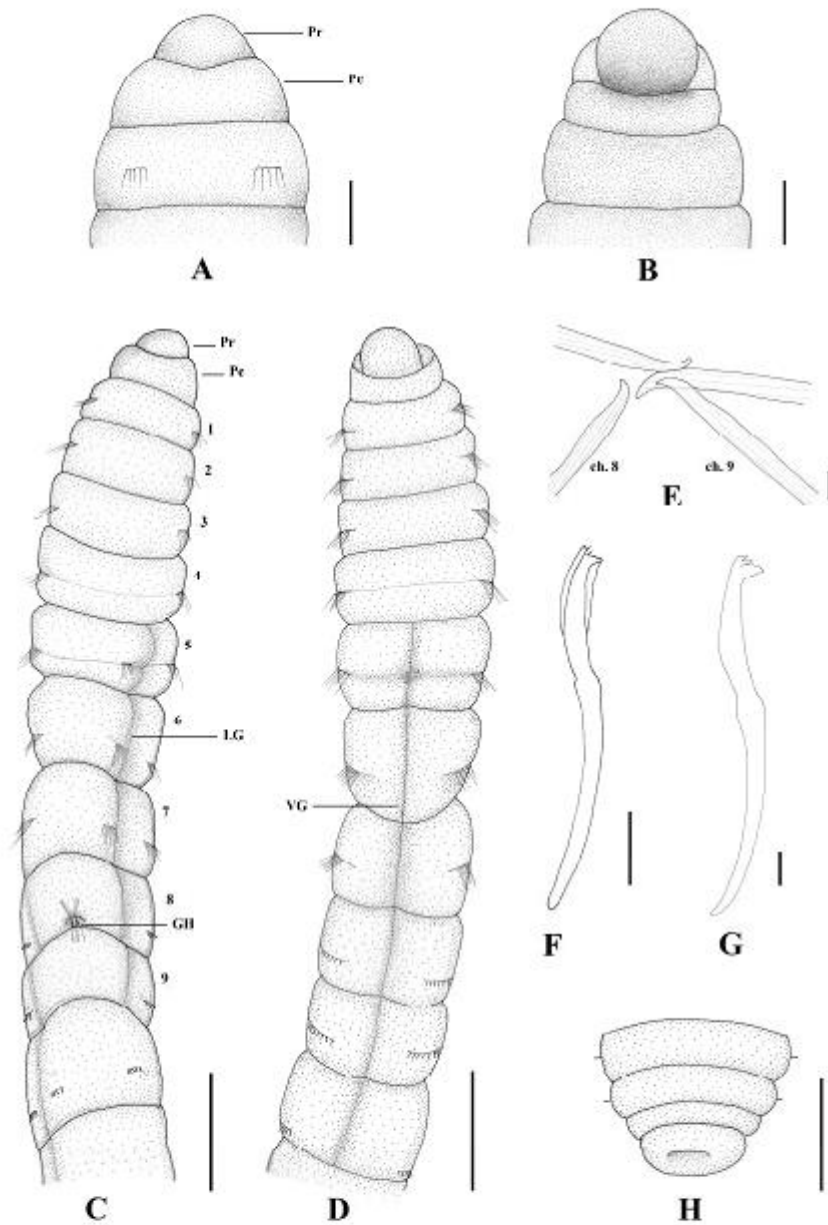
**Holotype:** São Paulo, Araçá Bay: ZUEC POL 16779: 23°48'55.1"S – 45°24'25.9"W; tidal flat; station 79(2)A; 07 May 2012; 1 spec.

**Paratypes:** São Paulo, Araçá Bay: ZUEC POL 17451 –paratype 1: 23°48'50.7"S – 45°24'28.0"W; tidal flat; station 51(3)A; 24 Feb 2012; 1 spec. ZUEC POL 17457 –paratypes 2 – 6: 23°48'36.1"S – 45°24'19.5"W; tidal flat; station 34(3)A; 29 Sep 2011; 5 specs. MNRJP 994 –paratype 7: 23°48'55.1"S – 45°24'25.9"W; tidal flat; station 79(2)A; 07 May 2012; 1 spec.

**Additional material examined (S1 Appendix):** São Paulo, Araçá Bay (25 specs.).

**Description.** Based on type material, additional material and specimens examined by SEM. Size range of material examined (complete individuals) 11.37–15.44 mm long, 0.45–0.6 mm wide and 50–62 chaetigers. Specimens slightly widest anteriorly, gradual narrowing posteriorly. Color in alcohol brownish. Prostomium conical, wider than longer (Figs 2A and 2C; 3A and 3D). Peristomium large, forming a complete achaetous ring, conspicuous dorsal and ventrally, wider than peristomium (Figs 2A and 2B; 3A and 3B); eyespots absent. Nuchal organs not visible using light microscopy or SEM. Chaetigers 1–4 similar, rectangular, weakly biannulate; chaetigers 5–9 similar, square, with mid-ventral and lateral groove (Figs 2C and 2D; 3D and 3E; 4A and 4B). Male and female adult specimens with unilimbate capillaries in notopodia and neuropodia of chaetigers 1–7, hooded hooks in neuropodia of chaetigers 8–9 and genital spines in notopodia of chaetigers 8–9. Notoetae arranged in a single row of 3–7 capillaries and 5–6 hooded hooks; neuroetae arranged in a single row of 3–8 capillaries and 4–6 hooded hooks. Thoracic hooded hooks with pointed, straight and short main fang, right angle with the shaft, surmounted by 2 apical teeth, one above the other; long curved shoulder; anterior shaft absent; developed node; long and slightly curved posterior shaft; short and smooth hood (Fig 2F). Chaetigers 8 and 9 with two straight genital spines with tips sharply curved and thin vertical grooves; spines of chaetiger 8 embedded and shorter than those of chaetiger 9; spines of chaetiger 9 external and larger than those of chaetiger 8 (Figs 2E; 3F and 4D). Division between thorax and abdomen not prominent (Figs 2C and 2D; 3D and 3E; 4A and 4B). Abdominal chaetigers as long as wide (Figs 2C and 3D); chaetigers with 8–9 hooded hooks in notopodia and 9–11 in neuropodia, reduced to one hook in far posterior; hooks slightly smaller than the thoracics with pointed, straight and short main fang, right angle with the shaft, protruding just slightly through frontal opening, surmounted by 2 teeth, one above the other; long and curved shoulder; anterior shaft absent; well-developed node; long and curved posterior shaft; long and smooth hood (Figs 2G; 3G and 4E). Branchiae absent. Pygidium a quite small simple lobe without anal cirri (Figs 2H and 3H).





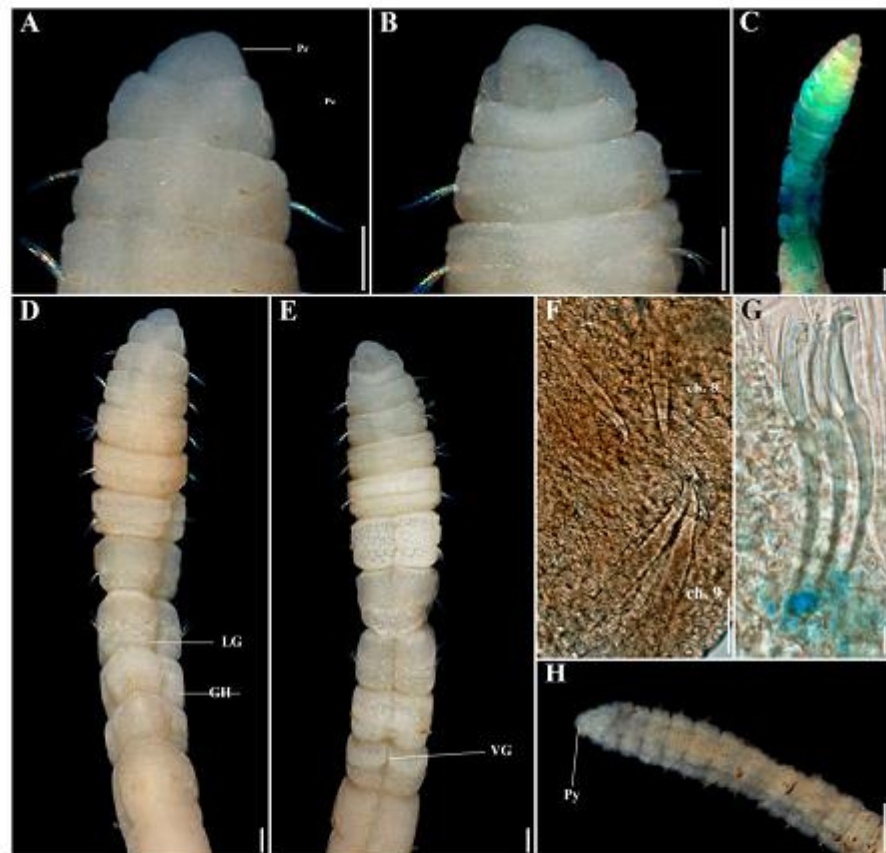
**Fig 2.** *Capitella aracaensis* sp. n. (A) Anterior end, dorsal view; (B) Anterior end, ventral view; (C) Thoracic region, dorsal view; (D) Thoracic region, ventral view; (E) Genital spines; (F) Thoracic hooded hook, lateral view; (G) Abdominal hooded hook, lateral view; (H) Abdominal end, ventral view.

hooded hook, lateral view; (H) Pygidium, dorsal view. Ch: chaetiger. GH: genital hook. LG: lateral groove. Pe: peristomium. Pr: prostomium. VG: ventral groove. Scale bars: A, B, 0.1 mm; C, D, 0.3 mm; E, F, 20  $\mu$ m; G, 10  $\mu$ m; H, 0.125 mm.

<https://doi.org/10.1371/journal.pone.0177760.g002>

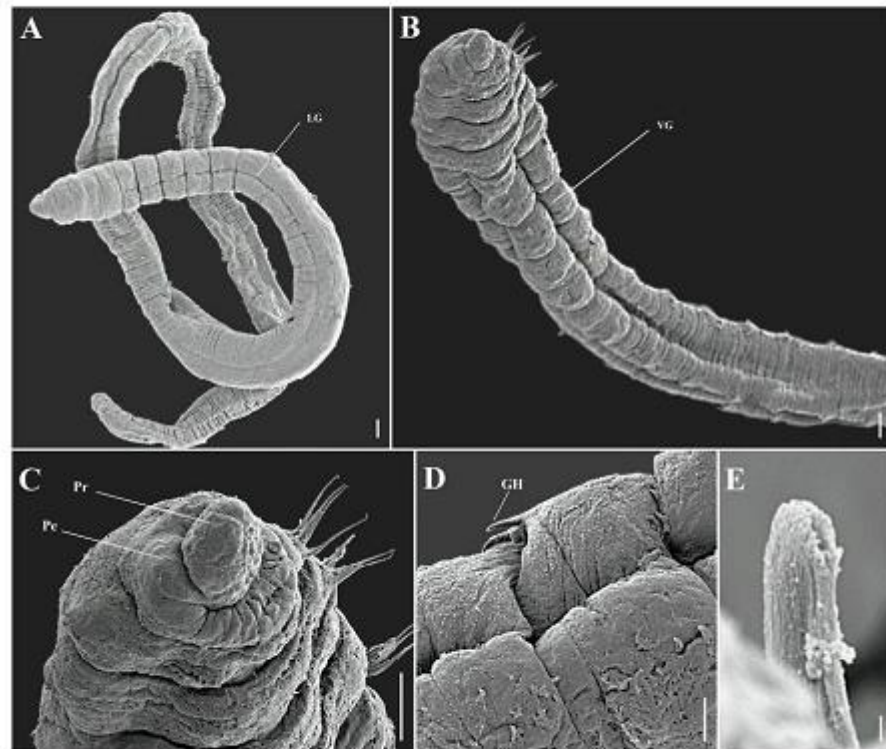
**Methyl green staining pattern.** Chaetigers 5–7 with a strip of small spots in the middle of the segment, chaetigers 8 and 9 darkly stained and abdominal segments staining uniformly (Fig 3C).

**Biology.** All specimens with genital spines. However, a few specimens presented oocytes inside the abdominal region, confirming they are female specimens.



**Fig 3. *Capitella aracaensis* sp. n.** (A) Anterior end, dorsal view; (B) Anterior end, ventral view; (C) Methyl green staining pattern; (D) Thoracic region, dorsal view; (E) Thoracic region, ventral view; (F) Genital spines; (G) Abdominal hooded hook, lateral view; (H) Posterior end and pygidium. Ch: chaetiger. GH: genital hook. LG: lateral groove. Pe: peristomium. Pr: prostomium. Py: pygidium. VG: ventral groove. Scale bars: A, B, D, E, F, H, 0.1 mm; C, 0.2 mm; G, 10  $\mu$ m.

<https://doi.org/10.1371/journal.pone.0177760.g003>

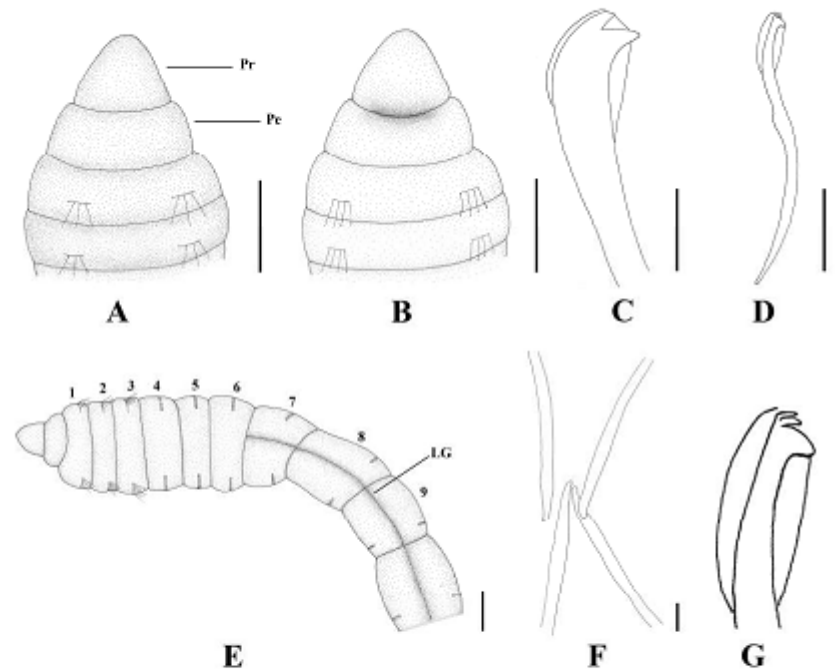


**Fig 4. *Capitella aracaensis* sp. n., SEM.** (A) Complete specimen; (B) Thoracic region, ventral view; (C) Anterior end, latero-ventral view; (D) Genital spines; (E) Abdominal hooded hook, frontal view. GH: genital hook. LG: lateral groove. Pe: peristomium. Pr: prostomium. VG: ventral groove. Scale bars: A, B, C, 0.1 mm; D, 0.5 mm; E, 1  $\mu$ m.

<https://doi.org/10.1371/journal.pone.0177760.g004>

**Remarks.** *Capitella aracaensis* sp. n., belongs to a group of species of *Capitella* with capillary chaetae on chaetigers 1–7 and hooded hooks on chaetigers 8 and 9. This group includes *C. amboensis*, *C. capitata*, *C. caribaeorum*, *C. dizonata*, *C. iatapiuna*, *C. perarmata*, *C. singularis* and *C. teleta*. *Capitella aracaensis* sp. n. shares some features with *C. caribaeorum*, *C. iatapiuna* and *C. singularis*, such as the peristomium forming a complete ring and the absence of eye-spots. These species differ, however, in the characteristics of their prostomium: in *C. caribaeorum* it is conical, with a dorsal depression and ventral groove; in *C. iatapiuna* it is quite rounded, as long as wide; and in *C. singularis* it is conical and smooth; while in *C. aracaensis* sp. n. it is rounded. The abdominal hooded hooks also differ in number and distribution of teeth above main fang: in *C. caribaeorum* there are several teeth arranged in four rows; in *C. iatapiuna* there are 5–6 teeth arranged in two rows on thoracic hooks and 8–9 teeth in three rows on abdominal hooks; and in *C. singularis* there are 11–14 teeth arranged in two rows; while in *C. aracaensis* sp. n. there are two teeth, one above the other, on both thoracic and abdominal hooded hooks. *Capitella capitata*, *C. dizonata*, *C. perarmata* and *C. teleta* differ





**Fig 5. *Capitella biota* sp. n.** (A) Anterior end, dorsal view; (B) Anterior end, ventral view; (C) Thoracic hooded hook, lateral view; (D) Abdominal hooded hook, lateral view; (E) Thoracic region, lateral view; (F) Genital spines; (G) Abdominal hooded hook, anterior end. Scale bars: A, B, E, 0.1 mm; C, 5  $\mu$ m; D, F, 15.6  $\mu$ m; G, 7.8  $\mu$ m.

<https://doi.org/10.1371/journal.pone.0177760.g005>

from *C. aracaensis* sp. n. by having a peristomium forming an incomplete ring and in features of the hooded hooks. *Capitella aracaensis* sp. n. can be distinguished by its rounded prostomium, large complete peristomium and hooded hooks with two teeth above the main fang, one above the other.

**Etymology.** This species was named after the Araçá Bay (São Sebastião, state of São), which has a high biodiversity [64] and is one of the samplings areas of this study.

**Habitat.** Intertidal region, in fine sand and mangrove.

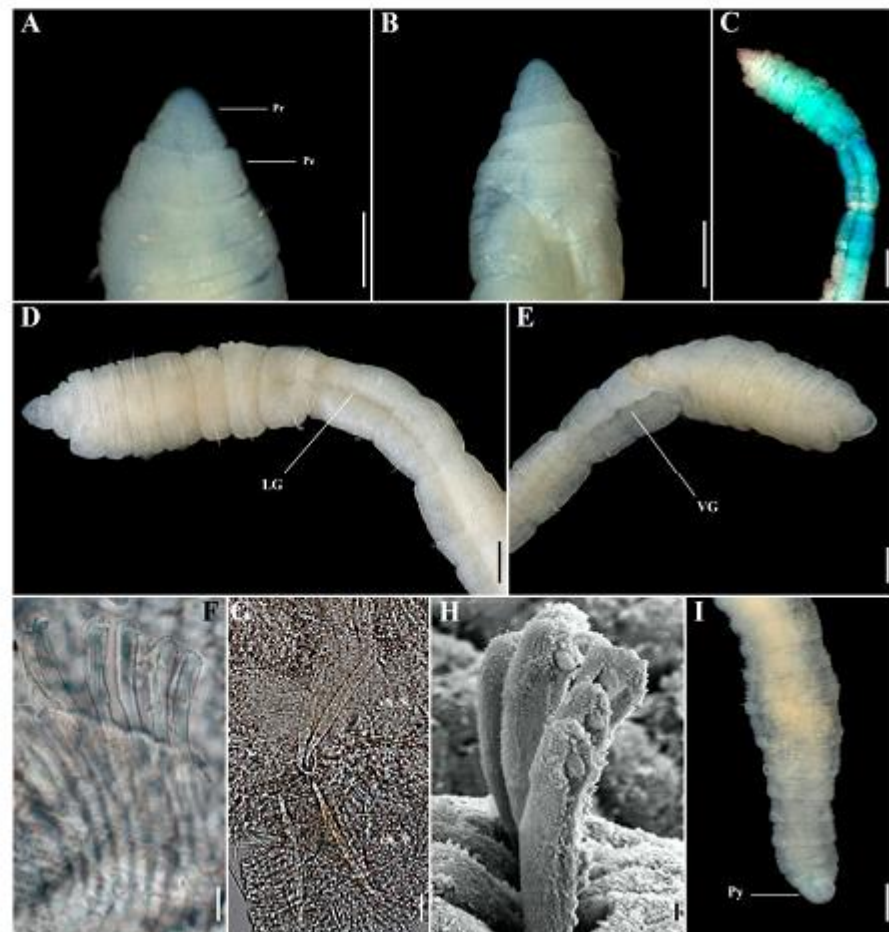
**Type locality.** Araçá Bay, São Sebastião, São Paulo, Brazil (South Atlantic Ocean).

**Distribution.** South Atlantic Ocean: Brazil (state of São Paulo).

***Capitella biota* sp. n. Silva & Amaral Figs 5 and 6.** urn:lsid:zoobank.org:act:66EA9584-1C4C-4074-A735-624A73806F72.

**Holotype:** São Paulo, Araçá Bay: ZUEC POL 16728: 23°48'51.4"S – 45°24'26.5"W; mangrove; station 62M; coll. 19 Mar 2014; 1 spec.

**Paratypes:** São Paulo, Araçá Bay: ZUEC POL 16729 –paratype 1: 23°48'51.4"S – 45°24'26.5"W; mangrove; station 62M; coll. 19 Mar 2014; 1 spec. MNRJP 997 –paratypes 2 – 3: 23°48'51.4"S – 45°24'26.5"W; mangrove; station 63M; coll. 19 Mar 2014; 2 specs. ZUEC POL 16731 –paratype 4: 23°48'51.4"S – 45°24'26.5"W; mangrove; station 144M; coll. 10 Jul 2014; 1



**Fig 6.** *Capitella biota* sp. n. (A) Anterior end, dorsal view; (B) Anterior end, ventral view; (C) Methyl green staining pattern; (D) Thoracic region, lateral view; (E) Thoracic region, ventral view; (F) Thoracic hooded hooks, lateral view; (G) Genital spines; (H) Abdominal hooded hooks, frontal view; (I) Posterior end and pygidium. Pr: prostomium. Py: pygidium. Scale bars: A, B, D, E, I, 0.1 mm; C, 0.2 mm; F, 5 µm; G, 20 µm; H, 1 µm.

<https://doi.org/10.1371/journal.pone.0177760.g006>

spec. ZUEC POL 16732—paratypes 5–7: 23°48'37.4"S – 45°24'21.4"W; tidal flat; station 117 (4); coll. 17 Sep 2013; 3 specs.

**Additional material examined** (S1 Appendix): São Paulo, Araçá Bay (141 specs.).

**Description.** Based on type material, additional material and specimens examined by SEM. Size range of material examined (complete individuals) 3.9–16.0 mm long (holotype 16.0 mm), 0.31–0.42 mm wide (holotype 0.4 mm) and 30–74 chaetigers (holotype 74 chaetigers). Body small, widest anteriorly, gradual narrowing posteriorly. Color in alcohol yellowish.

Prostomium pointed, longer than wider (Figs 5A and 5B; 6A and 6B). Peristomium distinct, forming a complete achaetous ring, conspicuous dorsal- and ventrally, wider than prostomium (Figs 5A and 5B; 6A and 6B); eyespots absent. Chaetigers 1–7 similar; chaetigers 8 and 9 slightly more narrow; all chaetigers with shallow intersegmental furrows; chaetigers 7–9 with a deep mid-ventral groove (Figs 5D and 6E) and a smooth lateral groove (Fig 6D). Adult specimens with unilimbate capillaries in notopodia and neuropodia of chaetigers 1–3 and hooded hooks in notopodia and neuropodia of chaetigers 4–9. Notochaetae arranged in a single row of 3–7 capillaries and 7–8 hooded hooks; neurochaetae arranged in a single row of 3–8 capillaries and 5–9 hooded hooks; chaetae emerging from the middle of the chaetigers (Figs 5D and 6D). Thoracic hooded hooks with pointed and short main fang, upward curved, surmounted by one apical tooth; long and slightly curved shoulder; long and smooth hood (Fig 6E). Division between thorax and abdomen not prominent (Figs 5D; 6D and 6E). Abdominal chaetigers as long as wide, anterior chaetigers with 6–9 hooded hooks in notopodia and 6–10 in neuropodia, reducing to 2–3 hooks in posterior chaetigers, emerging from the last third of the chaetigers. Hooded hooks small with rounded and robust main fang, right angle with the shaft, protruding through the frontal opening, surmounted by two teeth, one above the other; long and slightly curved shoulder; anterior shaft absent; developed node; long and well curved posterior shaft; long and smooth hood (Figs 5E; 6H). Chaetigers 8 and 9 with 2 embedded genital spines. Spines of chaetiger 8 thin, straight and with slightly curved tips; spines of chaetiger 9 larger than those of chaetiger 8, straight and with slightly curved tips (Figs 5E; 6G). Branchiae absent. Pygidium small simple lobe without anal cirri (Fig 6I).

**Methyl green staining pattern.** Chaetigers 7–9 and the first abdominal chaetiger darkly stained (Fig 6C).

**Remarks.** *Capitella biota* sp. n., belongs to a group of species of *Capitella* with capillary chaetae on chaetigers 1–3. This group includes *C. capitata tripartita*, *C. jonesi* and *C. minima tulearensis*. Although *C. jonesi* and *C. minima tulearensis* also have hooded hooks in noto- and neuropodia of chaetigers 4–9, the former has a peristomium forming an incomplete ring and eyespots, while the latter has hooded hooks with 7–9 teeth above main fang, distributed in three rows, both differing from *C. biota* sp. n. *Capitella capitata tripartita* differs from *C. biota* sp. n. in having eyespots and mixed capillary chaetae and hooded hooks in noto- and neuropodia of chaetigers 4–9. *Capitella biota* sp. n. can be distinguished by having a peristomium forming a complete ring and thoracic hooded hooks with one apical tooth and abdominal hooks with two teeth above main fang, one above the other.

**Etymology.** This species was named after the “BIOTA-FAPESP Program”, which allowed the realization of the “BIOTA-Araçá Project”, responsible for funding the collection of most of the individuals reported in this paper.

**Habitat.** Intertidal region, in fine sand and mangrove sediments.

**Type locality.** Aracá Bay, São Sebastião, São Paulo, Brazil (South Atlantic Ocean).

**Distribution.** South Atlantic Ocean: Brazil (states of Bahia and São Paulo).

*Capitella neoaciculata* sp. n. Silva & Seixas Figs 7–9. urn:lsid:zoobank.org:act:138592D0-D7E3-4C5D-9DB4-6F2E009A73E9.

**Holotype:** São Paulo, Aracá Bay: ZUEC POL 16816: 23°48′51.4″S – 45°24′26.5″W; mangrove; station 205M; coll. 17 Mar 2015; 1 spec.

**Paratypes:** São Paulo, Aracá Bay: ZUEC POL 17399: 23°48′46.6″S – 45°24′29.8″W; mangrove; station 100M; coll. 10 Jul 2014; 2 specs. ZUEC POL 17384: 23°48′51.4″S – 45°24′26.5″W; mangrove; station 137M; coll. 10 Jul 2014; 1 spec. MNRJ 1429: 23°48′51.4″S – 45°24′26.5″W; mangrove; station 205M; coll. 17 Mar 2015; 6 specs.

**Additional material examined (S1 Appendix):** São Paulo, Aracá Bay (655 specs.); Rio de Janeiro, Piratininga Lagoon (34 specs.); Rio de Janeiro, Itaipu Lagoon (45 specs.).

**Description.** Based on type material, additional material and specimens examined by SEM. Size range of material examined (complete individuals) 5.0–22.0 mm long, 0.2–1.2 mm wide and 30–73 chaetigers. Specimens widest anteriorly, gradual narrowing posteriorly (Fig 7C). Color in alcohol yellowish. Prostomium triangular, wider than longer, with a deep dorsal depression (Figs 7A and 7C; 8A; 9A and 9D) and a slight ventral groove (Figs 7B; 8B and 9E). Peristomium forming an incomplete achaetous ring, slightly conspicuous dorsal and laterally (Figs 7A, 7C and 7D; 8B, 8D and 8E; 9A); similar in width to the first chaetiger; eyespots absent. Chaetiger 1 the smallest; chaetigers 2–4 and 7 similar in width and length; chaetigers 5 and 6 wider and longer; chaetigers 8 and 9 rectangular in female, rounded in male; all chaetigers with shallow intersegmental furrows; chaetigers 5–9 with mid-ventral and lateral groove (Figs 7D; 8D and 8E; 9D and 9E). Adult female specimens with capillaries in notopodia and neuropodia of chaetigers 1–8, hooded hooks in notopodia and neuropodia of chaetiger 9, and a pair of genital spines in neuropodia of chaetiger 9. Male adult specimens with acicular spines in notopodia and neuropodia of chaetigers 1 and 2 (Figs 7A–7D; 8C; 9A and 9D), capillaries in notopodia and neuropodia of chaetigers 3–7, capillaries in neuropodia of chaetiger 8, hooded hooks in neuropodia of chaetiger 9, and genital spines in notopodia of chaetigers 8 and 9. Noto- and neuroaciculae arranged in a single row of 2 or 3 acicular spines; noto- and neurosetae arranged in a single row of 5–13 unilimbate capillaries and 6–10 hooded hooks; chaetae emerging from the last third of the chaetiger. Thoracic hooded hooks with a rounded, thick and slightly curved main fang, surmounted by six apical teeth arranged in two rows (3 basally and 3 in superior row); long straight shoulder; short anterior shaft; inconspicuous node; long posterior shaft; long and smooth hood (Figs 7E and 7F; 8E and 9F). In females, chaetiger 9 with a pair of small, thin and slightly curved genital spines. In males, chaetiger 8 with 6 falcate external genital spines (2 fascicles) with tips sharply curved, narrower than those of chaetiger 9; chaetiger 9 with 6 straight embedded genital spines (2 fascicles), curved apically, with blunt tips, larger than those of chaetiger 8 (Figs 8I and 9C). Division between thorax and abdomen prominent. Abdominal chaetigers as long as wide; chaetigers with 3–10 hooded hooks in notopodia and 5–15 in neuropodia, reducing to 4 notopodial and 7 neuropodial hooks. Abdominal hooded hooks shorter than the thoracics with a pointed, thin and slightly curved main fang, surmounted by six teeth arranged in two rows (3 basally and 3 on superior row); long and slightly curved shoulder, short anterior shaft; developed node; long posterior shaft; short and smooth hood (Figs 7G and 7H; 8G, 8H and 8I and 9G). Ventral depression along the abdominal region (Figs 8K and 9H). Neuropodial hooded hooks emerging from long parapodial ridges in far posterior chaetigers (Figs 8K and 9H). Branchiae absent. Pygidium a large simple lobe, fused dorsally and slightly bilobate ventrally (heart-shaped), without anal cirri (Figs 7I and 7J; 8K and 9H).

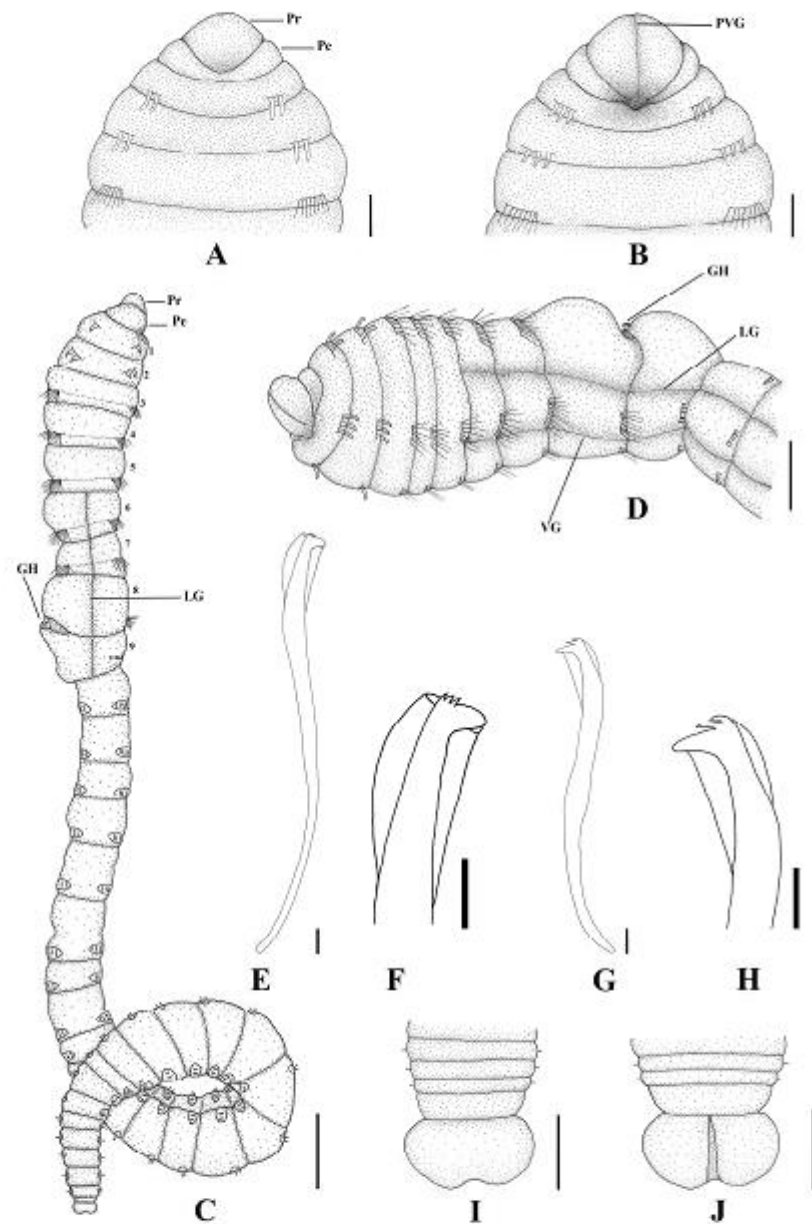
**Methyl green staining pattern.** Specimens staining uniformly, except the two first chaetigers which are lightly speckled (Fig 8C).

**Variation.** The presence of the acicular spines varies according to the length and perhaps sexual development of the specimens. Large females (more than 0.8 mm wide) can have acicular spines in notopodia of chaetiger 1, however the aciculae are less developed than in males; and small males, with genital spines less developed, commonly lack acicular spines in neuropodia of chaetigers 1 and 2. Larger specimens can also have capillary chaetae on chaetiger 9.

**Biology.** Almost all specimens with genital spines; specimens with small and less developed genital spines only on chaetiger 9 presented oocytes inside the abdominal region, confirming they are female specimens. Males, with robust genital spines, did not present oocytes.

**Remarks.** *Capitella neoaciculata* sp. n., belongs to a group of species of *Capitella* with capillary chaetae on chaetigers 1–8 and hooded hooks on chaetiger 9. This group includes *C. aciculata* and *C. teres*. *Capitella neoaciculata* sp. n. differs from *C. teres* by having acicular spines in



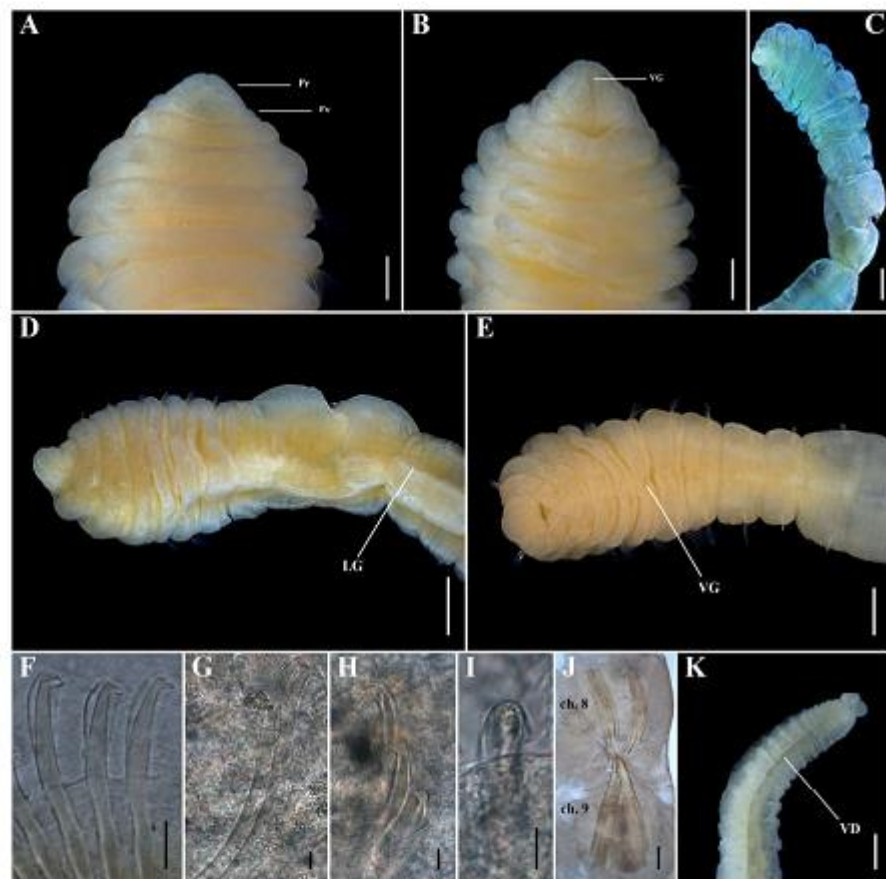


**Fig 7. *Capitella neoaciculata* sp. n.** (A) Anterior end, dorsal view; (B) Anterior end, ventral view; (C) Complete male specimen, lateral view; (D) Thoracic region of a male, lateral view; (E) Thoracic hooded hook, lateral view; (F) Thoracic hooded hook, lateral view; (G) Thoracic hooded hook, lateral view; (H) Thoracic hooded hook, lateral view; (I) Thoracic hooded hook, lateral view; (J) Thoracic hooded hook, lateral view.

Thoracic hooded hook, anterior end; (G) Abdominal hooded hook, lateral view; (H) Abdominal hooded hook, anterior end; (I) Pygidium, dorsal view; (J) Pygidium, ventral view. GH: genital hook. LG: lateral groove. Pe: peristomium. Pr: prostomium. PVG: prostomial ventral groove. VG: ventral groove. Scale bars: A, B, D, 0.2 mm; C, 0.5 mm; E, F, G, H, 5  $\mu$ m; I, J, 0.125 mm.

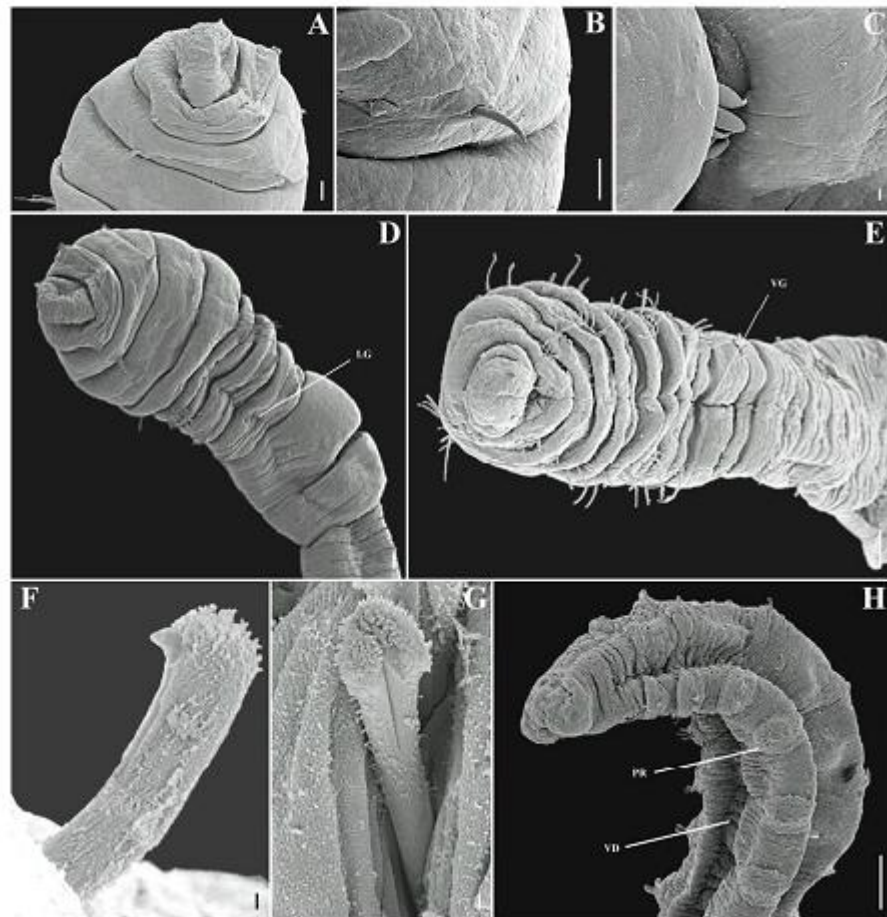
<https://doi.org/10.1371/journal.pone.0177760.g007>

the first chaetigers. Besides the presence of acicular spines, *Capitella neoaciculata* sp. n. differs from *C. aciculata*, in that females lack acicular spines, in the notopodia of chaetigers 1 and 2, hooded hooks have six teeth arranged in two rows (3 basally and 3 on superior row), rather



**Fig 8. *Capitella neoaciculata* sp. n.** (A) Anterior end, dorsal view; (B) Anterior end, ventral view; (C) Methyl green staining pattern; (D) Thoracic region of a male, lateral view; (E) Thoracic region of a male, ventral view; (F) Thoracic hooded hook, lateral view; (G, H) Abdominal hooded hook, lateral view; (I) Abdominal hooded hook, frontal view; (J) Genital spines; (K) Posterior end and pygidium. Ch: chaetiger. LG: lateral groove. Pe: peristomium. Pr: prostomium. VD: ventral depression. VG: ventral groove. Scale bars: A, B, D, E, K, 0.2 mm; C, 0.5 mm; F, G, H, I, 5  $\mu$ m; J, 0.1 mm.

<https://doi.org/10.1371/journal.pone.0177760.g008>



**Fig 9.** *Capitella neoaciculata* sp. n., SEM. (A) Anterior and lateral view; (B) Acicular spine; (C) Genital spines; (D) Thoracic region of a male, lateral view; (E) Thoracic region of a male, ventral view; (F) Thoracic hooded hook, lateral view; (G) Abdominal hooded hook, lateral view; (H) Posterior end and pygidium. LG: lateral groove. PR: parapodial ridges. VD: ventral depression. Scale bars: A, B, D, E, H, 0.1 mm; C, 10 µm; F, G, 1 µm.

<https://doi.org/10.1371/journal.pone.0177760.g009>

than five teeth (3 basally and 2 on superior row), there is a ventral depression along the abdominal region, and the pygidium is large and heart-shaped rather than inconspicuous and a simple ring as in *C. aciculata*.

**Etymology.** This species was named based on the presence of acicular spines as *Capitella aciculata*, however, with a new combination of characters.

**Habitat.** From intertidal region to shallow subtidal regions (up to 0.5 m), in fine sand.

**Type locality.** Aracá Bay, São Sebastião, São Paulo, Brazil (South Atlantic Ocean).

**Distribution.** South Atlantic Ocean: Brazil (states of São Paulo, Rio de Janeiro and Pernambuco).

*Capitella nonatoi* sp. n. Silva & Amaral Figs 10–12. urn:lsid:zoobank.org:act:6F87DE88-1079-41A9-8A1C-780006A46538.

**Holotype:** São Paulo, Araçá Bay: ZUEC POL 17585: 23°48'39.4"S – 45°24'26.2"W; tidal flat; station 77(2); coll. 24 Jun 2013; 1 spec.

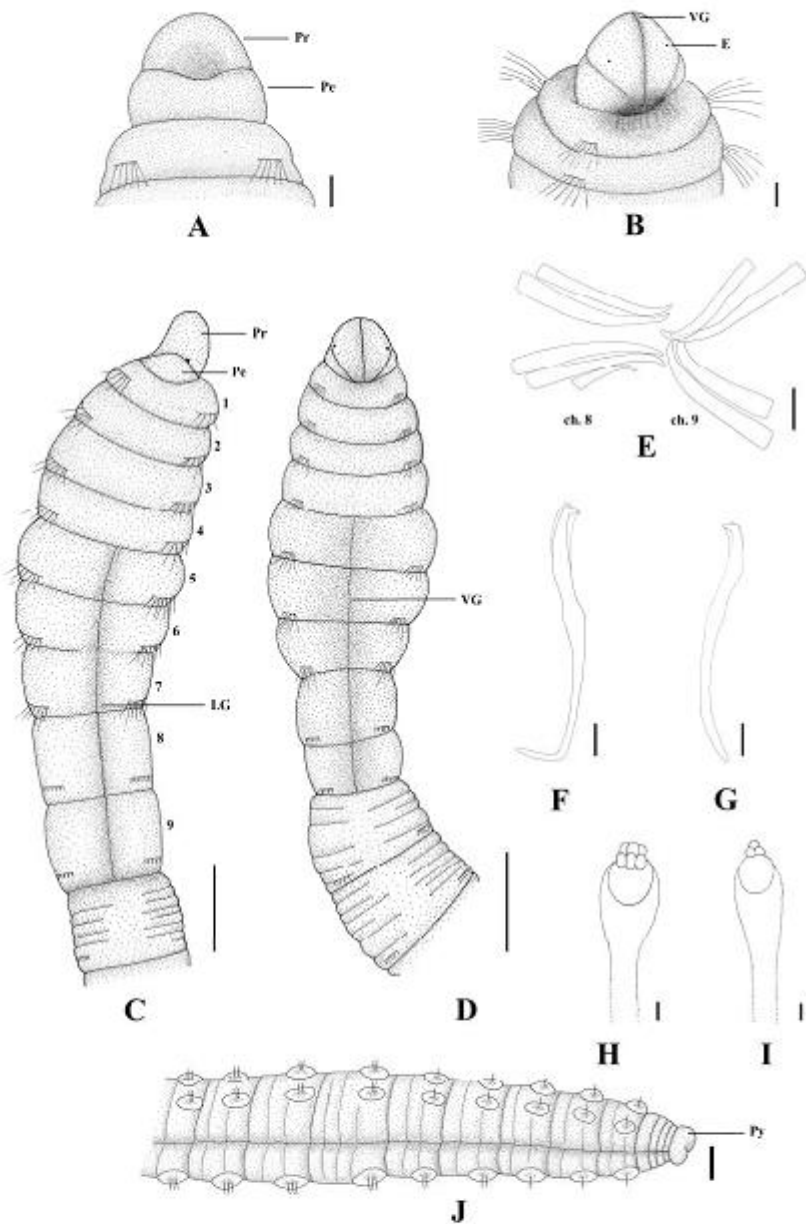
**Paratypes:** São Paulo, Araçá Bay: ZUEC POL 17460 –paratypes 1 – 3: 23°48'37.4"S – 45°24'21.4"W; tidal flat; station 80(1); coll. 24 Jun 2013; 3 specs. ZUEC POL 17461 –paratypes 4 – 8: 23°48'37.4"S – 45°24'21.4"W; tidal flat; station 6(1); coll. 13 Oct 2012; 5 specs. MNRJP 995 –paratypes 9 – 11: 23°48'51.4"S – 45°24'26.5"W; mangrove; station 124M; coll. 10 Jul 2014; 3 specs. MNRJP 996 –paratypes 12 – 15: 23°48'37.4"S – 45°24'21.4"W; tidal flat; station 80(4); coll. 24 Jun 2013; 4 specs.

**Additional material examined (S1 Appendix):** São Paulo, Araçá Bay (3,463 specs.); Rio de Janeiro, Itaipú Lagoon (15 specs.); Paraná, Paranaguá Bay (2 specs.); Pará, Caeté Bay (11 specs.).

**Description.** Based on type material, additional material and specimens examined by SEM. Size range of material examined (complete individuals) 3.1–15.0 mm long (holotype 7.5 mm), 0.3–0.7 mm wide (holotype 0.45 mm) and 31–71 chaetigers (holotype 49 chaetigers). Specimens widest anteriorly, gradual narrowing posteriorly. Color in alcohol yellowish. Prostomium short, rounded, as wide as long, with a dorsal smooth depression (Figs 10A; 11A and 12A) and a ventral groove (Figs 10B; 11B and 12B). Peristomium forming an incomplete achaetous ring, conspicuous dorsal and laterally (Figs 10A and 10B; 11A and 11B), similar width than prostomium; eyespots present, a latero-ventral reddish spot (Figs 10B and 11B). Nuchal organ visible (Fig 12B). Chaetigers 1–5 gradually increasing in size, chaetiger 5 the largest; chaetigers 6–7 gradually decreasing in size; chaetigers 8 and 9 quadrangular in females and rounded in males; chaetigers 5–9 with mid-ventral and lateral groove (Figs 10C and 10D; 11D, 11E and 11F; 12D and 12E). Adult specimens with unilimbate capillaries (Fig 12C) in notopodia and neuropodia of chaetigers 1–7 and hooded hooks in notopodia and neuropodia of chaetigers 8–9. Notoetae arranged in a single irregular row of 3–15 capillaries and 5–8 hooded hooks; neuroetae arranged in a single irregular row of 4–17 capillaries and 5–8 hooded hooks. Thoracic hooded hooks with a pointed and large main fang at a right angle to the shaft, surmounted by 6 apical teeth arranged in two rows (3 basally and 3 in superior row); short anterior shaft; developed node; long and curved posterior shaft; short and smooth hood (Figs 10F and 10H; 11H and 11I and 12G). All chaetae emerging from the last third of the chaetiger. Chaetiger 8 with 4 straight external genital spines (2 fascicles), with tips sharply curved, narrower and shorter than those of chaetiger 9; chaetiger 9 with 4 straight embedded genital spines (2 fascicles), with tips sharply curved, wider and larger than those of chaetiger 8 (Figs 11G; 12E and 12F). Division between thorax and abdomen prominent. Abdominal chaetigers as long as wide; chaetigers with 2–5 hooded hooks in notopodia and 4–10 in neuropodia, reducing to 2 hooded hooks in notopodia and 4 in neuropodia. In far posterior chaetigers, neuropodial hooded hooks emerging from parapodial ridges (Figs 10I and 11I). Abdominal hooded hooks smaller than the thoracics, with a pointed main fang, upward curved, surmounted by three teeth arranged in two rows (2 basally and 1 on superior row); short shaft; developed node; long and curved posterior shaft; short and smooth hood (Figs 10G and 10I; 11I and 11K and 12H). Branchiae absent. Pygidium a small simple lobe without anal cirri (Figs 10J and 11J).

**Methyl green staining pattern.** Chaetigers 5–8 darkly stained, chaetigers 8 and 9 and the first two abdominal with dark speckles (Fig 11C).

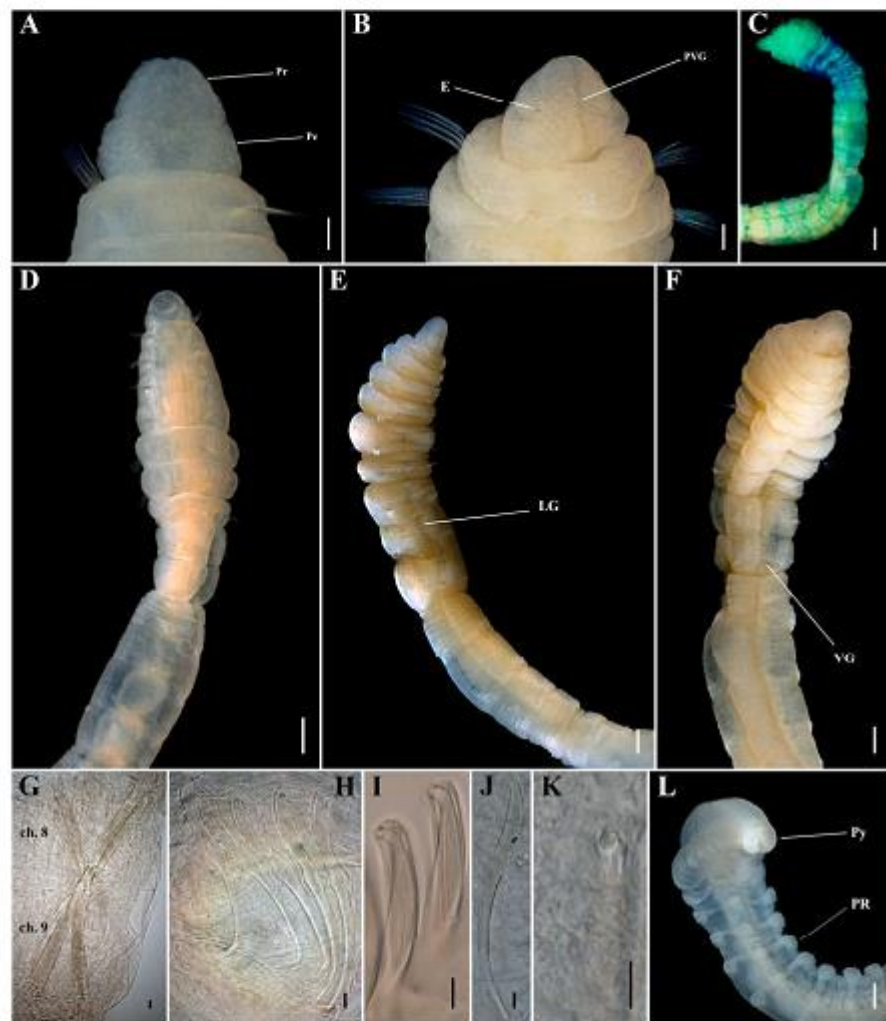




**Fig 10.** *Capitella nonatoi* sp. n. (A) Anterior end, dorsal view; (B) Anterior end, ventral view; (C) Thoracic region of a female specimen, lateral view; (D) Thoracic region of a female specimen, ventral view; (E) Genital hooks; (F) Thoracic

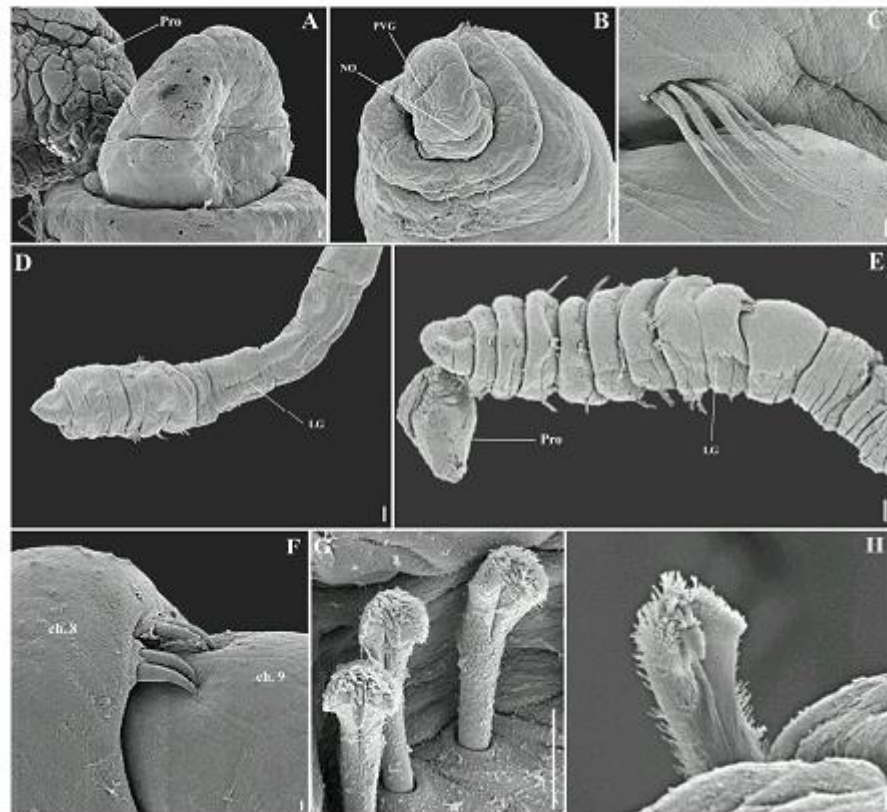
hooded hook, lateral view; (G) Abdominal hooded hook, lateral view; (H) Thoracic hooded hook, frontal view; (I) Abdominal hooded hook, frontal view; (J) Posterior end with pygidium, dorso-lateral view. E: eyespot. LG: lateral groove. Pe: peristomium. Pr: prostomium. PVG: prostomial ventral groove. Py: pygidium. VG: ventral groove. Scale bars: A, B, 0.01 mm; C, D, 1 mm; E, 0.125 mm; F, G, 10  $\mu$ m; H, I, 1  $\mu$ m; J, 0.1 mm.

<https://doi.org/10.1371/journal.pone.0177760.g010>



**Fig 11. *Capitella nonatoi* sp. n.** (A) Anterior end, dorsal view; (B) Anterior end, ventral view; (C) Methyl green staining pattern; (D) Thoracic region of a male specimen, dorsal view; (E) Thoracic region of a male specimen, lateral view; (F) Thoracic region of a male specimen, ventral view; (G) Genital hooks; (H) Thoracic hooded hook, lateral view; (I) Thoracic hooded hook, frontal view; (J) Abdominal hooded hook, lateral view; (K) Abdominal hooded hook, frontal view; (L) Posterior end and pygidium. Ch: chaetiger. E: eyespot. Pe: peristomium. Pr: prostomium. LG: lateral groove. VG: ventral groove. PVG: prostomial ventral groove. PR: parapodial ridges. Scale bars: A, B, F, L, 0.1 mm; C, D, E, 0.2 mm; G, 20  $\mu$ m; H, I, J, K, 5  $\mu$ m.

<https://doi.org/10.1371/journal.pone.0177760.g011>



**Fig 12. *Capitella nonatoi* sp. n., SEM.** (A) Anterior end, dorso-lateral view; (B) Anterior end, ventro-lateral view; (C) Capillary chaetae; (D) Thoracic region of a female specimen, lateral view; (E) Thoracic region of a male specimen, lateral view; (F) Genital hooks; (G) Thoracic hooded hooks, frontal view; (H) Abdominal hooded hook, frontal view. Ch: chaetiger. LG: lateral groove. NO: nuchal organ. PVG: prostomial ventral groove. Pro: proboscis. Scale bars: A, C, F, 10  $\mu$ m; B, D, E, 0.1 mm; G, H, 1  $\mu$ m.

<https://doi.org/10.1371/journal.pone.0177760.g012>

**Biology.** All specimens without genital spines, with oocytes in the abdominal region confirming they are female specimens.

**Remarks.** *Capitella nonatoi* sp. n. belongs to a group of species of *Capitella* with capillary chaetae on chaetigers 1–7 and hooded hooks on chaetigers 8 and 9. *Capitella teleta* resembles *C. nonatoi* sp. n. in the overall body shape, peristomium forming an incomplete ring, a very conspicuous nuchal organ and presence of eyespots. However, the former differs from *C. nonatoi* sp. n. in having a flattened and long prostomium, while in *C. nonatoi* sp. n. the prostomium is short and rounded. The differences are also related to the number and features of the abdominal hooded hooks and number of genital spines. *Capitella teleta* has 4–6 hooks in notopodia and 5–6 in neuropodia, with six teeth above main fang, while *C. nonatoi* sp. n. has 2–5 hooded hooks in notopodia and 4–10 in neuropodia, with three teeth arranged in two

rows. Furthermore, *C. teleta* has 6–8 genital spines on chaetiger 8 and *C. nonatoi* sp. n. has four. *Capitella caribaeorum*, *C. iatapiauna* and *C. singularis* differ from *C. nonatoi* sp. n. by having a peristomium forming a complete ring and the absence of eyespots. Besides *C. perarmata*, *C. capitata* and *C. dizonata* also have a peristomium forming an incomplete ring but they differ from *C. nonatoi* sp. n. by the absence of eyespots and features of the hooded hooks such as number of teeth and their distribution above main fang. *Capitella nonatoi* sp. n. can be distinguished by its short and rounded prostomium with a dorsal smooth depression and a ventral groove, and eyespots.

**Etymology.** This species was named *in memoriam* of Professor Edmundo Ferraz Nonato, who was the pioneer on taxonomy of Polychaeta in Brazil and who had influenced many researchers with his dedication, knowledge and passion for polychaetes and science.

**Habitat.** Intertidal region, in fine sand and mangrove sediments.

**Type locality.** Aracá Bay, São Sebastião, São Paulo, Brazil (South Atlantic Ocean).

**Distribution.** South Atlantic Ocean: Brazil (states of Bahia, Pará, Paraná, Pernambuco, Rio de Janeiro and São Paulo).

### Molecular analysis

The phylogenetic analyses were based on 134 sequences of 16S and 106 of COI. The final alignment consisted of 558 bp for 16S and 655 bp for COI. Excluding the outgroup species, the 16S sequence had 215 (38.5%) variable sites, while the COI sequence had 273 (41.7%). The maximum likelihood and Bayesian inference method recovered the same species, as had been previously identified here by morphological characteristics (*C. aracaensis* sp. n., *C. biota* sp. n., *C. neoaciculata* sp. n. and *C. nonatoi* sp. n.). The four species clades were reciprocally monophyletic and were well supported in all datasets (COI, 16S and concatenated) and recovery methods (Fig 13 and S1 Fig). The *Capitella nonatoi* sp. n. clade included individuals from 12 sampling sites, while *C. neoaciculata* sp. n. was present in 6 sites, *C. biota* sp. n. in two sites and *C. aracaensis* sp. n. in only one site.

The mean pairwise genetic distance within species based on the K2P ranged from 0% (*C. aracaensis* sp. n.) to 0.5% (*C. neoaciculata* sp. n.) in 16S and from 0% (*C. aracaensis* sp. n.) to 2.3% (*C. biota* sp. n.) in COI (Table 2). In relation to the genetic distance among the *Capitella* species, the K2P values ranged from 24.4% (*C. nonatoi* sp. n. × *C. neoaciculata* sp. n.) to 46.4% (*C. aracaensis* sp. n. × *C. neoaciculata* sp. n.) in 16S and from 15.7% (*C. aracaensis* sp. n. × *C. neoaciculata* sp. n.) to 26.5% (*C. nonatoi* sp. n. × *C. capitata*) in COI (Table 2). As expected, the values based on *p*-distance were lower than the K2P (S1 Table).

### Discussion

The use of mtDNA sequences for species identification may speed up global diversity estimations [65], while the robustness of the morphological information improves the quantity and quality of species descriptions, as well as the understanding of the morphological adaptations in their evolutionary history [66–67]. Our morphological and molecular data were congruent and supported the existence of four different species of *Capitella*, all of them rather distinct from *Capitella capitata*, increasing the biodiversity of the genus along the Brazilian coast.

The delimitation of the boundaries of the various *Capitella* species along Brazilian waters has revealed a hidden local diversity and may enhance assessments of environmental health [68] as an indicator of organic pollution [69–72]. Sympatric occurrences were observed in six sites; most species distributed along the coast, while one only at only one site (*Capitella aracaensis* sp. n.). Combined morphological and molecular approaches were also conducted and have yielded similar results [25–26, 73–74]. Indeed, these authors identified distinct species







The scale bar represents the average nucleotide substitutions per site. Brown arrow shows the *Capitella capitata* from the type-locality (west Greenland). Gray boxes indicate Genbank sequences from *Capitella* species. Asterisks (\*) indicates that the support value was lower than 70 (ML) or 0.7 (BI), and a dash (-) indicates that the branch was not recovered.

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among the species complexes, increasing the richness of the target genus [25, 26, 73, 74]. Interestingly, the taxonomic confusion surrounding *Capitella* species can be illustrated through a number of studies that focused on the genus, but were unable to identify specimens at the species level [75–79].

Our results indicate that many studies may have overlooked the richness of *Capitella* species due to the challenges in the morphological taxonomic identification, despite the ecological importance of the genus [7]. The four new species described here were morphologically differentiated from all congeners based mainly on the shape of the prostomium, peristomium and thorax, as well as the shape and number of thoracic and abdominal hooded hooks, the shape of the pygidium and of the genital hooks. The differences of these characteristics among the *Capitella* species are summarized in a table in [5]. Although the morphology of the prostomium and peristomium may be affected by distinct fixation protocols [80], they were very informative characteristics and should be explored, as they are quite variable and diagnostic for species within the genus [3–6, 59]. We would like to highlight the fact that these new species were distinguished initially by a morphological analysis. The sequence data just confirmed our findings and helped to support the specific status of the recognized morphospecies.

Among these new species, the most widespread and abundant was *C. nonatoi* sp. n., with records in all sampled sites along 4,500 Km along the Brazilian coast, from the North (Pará) to the South (Paraná). Due to its high abundance and wide distribution, this species has likely been misidentified as *C. capitata* by previous studies [81–84]. The second most widespread species was *C. biota* sp. n., which was reported from the Northeast (Bahia) to the Southeast (São Paulo), ranging over an area of 1,630 Km. *Capitella aracaensis* sp. n. was more rare in our sampling, with low abundances and was restricted to one site of the Southeast Brazil (São Paulo). We suggested that this species is rare because Araçá Bay [64], the type locality, was exhaustively and systematically sampled over a large area during the four years of the BIOTA-FAPESP Program (Thematic Grants, Process 2011/50317-5). During these four years we found just a few specimens of *Capitella aracaensis* sp. n., meanwhile the other three species were frequent and abundant at the same site. The discrepancies regarding the geographic distributions of these species could be explained by sampling effort, biological aspects such as their differing reproduction strategies [85], larvae development, colonization capacity [86], and physiological tolerances [12].

**Table 2. Intraspecific (in bold) and interspecific mean pairwise genetic distance based on Kimura-2-parameters (K2P) for 16S (on top) and COI (below). All values are in percentage. Number in parenthesis represents the standard error.**

|                                  | 1                | 2                | 3                | 4                | 5                |
|----------------------------------|------------------|------------------|------------------|------------------|------------------|
| 1— <i>C. nonatoi</i> sp. n.      | <b>0.4 (0.2)</b> |                  |                  |                  |                  |
|                                  | <b>1.6 (0.5)</b> |                  |                  |                  |                  |
| 2— <i>C. neoaciculata</i> sp. n. | 25.4 (3.3)       | <b>0.4 (0.2)</b> |                  |                  |                  |
|                                  | 21.5 (3.3)       | <b>1.7 (0.5)</b> |                  |                  |                  |
| 3— <i>C. biota</i> sp. n.        | 31.8 (3.7)       | 35.9 (4.1)       | <b>0.4 (0.2)</b> |                  |                  |
|                                  | 17.9 (2.9)       | 20.5 (3.2)       | <b>2.2 (0.6)</b> |                  |                  |
| 4— <i>C. aracaensis</i> sp. n.   | 33.6 (4.0)       | 46.4 (5.3)       | 32.8 (3.8)       | <b>0.0 (0.0)</b> |                  |
|                                  | 20.6 (3.2)       | 15.7 (2.7)       | 20.3 (3.2)       | <b>0.0 (0.0)</b> |                  |
| 5— <i>C. capitata</i>            | 26.0 (3.5)       | 29.6 (3.8)       | 31.7 (3.9)       | 35.7 (4.4)       | <b>nd</b>        |
|                                  | 26.5 (3.9)       | 19.0 (3.1)       | 22.9 (3.4)       | 21.2 (3.4)       | <b>1.7 (0.7)</b> |

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The low levels of intraspecific molecular variations recorded for all the four *Capitella* species (with a maximum of 2.2% for *C. biota* sp. n.) were expected within the same lineage. Usually values lower than 6% indicate that lineages are not composed of some sub-complex of the species [74]. On the other hand, high interspecific variation, as was observed here, 15.7% up to 26.5% for COI, and 24.4% up to 46.4% for 16S was observed in other congeners, including between the deep-sea species, for example *C. iatapiuna* and *C. teleta* (20% for 16S) [5]. A similar molecular interspecific variation was found for errant [24, 87], and sedentary annelids [27]. The COI values were compatible with the 10-fold rule of species delimitation [88], supporting the hypothesis that the sample groups indeed represent four different species. Therefore, no less conservative method of species delimitation based on cluster recognition [89] was necessary to confirm this status. Recently, COI sequences have been applied to clarify the systematics of the *Capitella capitata* complex [90–91]. A similar result was found in the Gulf of Mexico, with two species under the name *C. capitata*, with 21.7% of K2P divergence [90], while [91] found the same haplotype of *C. teleta* both on the Atlantic coast of USA and Japan. Despite the fact that these two studies are inconclusive regarding their morphological approaches, intermediate morphologies between the species [90] and an intraspecific variability within the species [91] were found.

Thus, considering the intra and inter-specific variation calculated for these particular mitochondrial genes, we concluded that these divergences support the distinction among the four morphologically delimited species. Furthermore, a genetic distance comparison showed that the COI values were higher than 16S values when considering intraspecific comparisons. However, in relation to the differences between species, the COI values were smaller than the 16S values.

In addition to morphological and molecular studies confirming the cosmopolitan status of some species [92–93], our findings support restricting the geographical range of *C. capitata*, as was previously proposed by Blake [3]. Furthermore, we revealed a hidden diversity within this genus along the Brazilian coast. Despite the fragmentation of the *C. capitata* complex in at least four species, this does not indicate that each species has a restricted distribution, as frequently expected in this type of study. Thus, even for marine invertebrates with a supposedly low potential for dispersion, the population cohesion along a wide distribution can be maintained.

Finally, this study showed the importance of exhaustive and systematic sampling to unveil cryptic and rare species. Furthermore, this result emphasized the effectiveness of careful and accurate morphological study as a tool to differentiate species of *Capitella*.

## Supporting information

**S1 Appendix. Material examined.** List of additional material examined of *Capitella* species. (DOCX)

**S1 Fig. Phylogenetic tree based on maximum likelihood for concatenated dataset (16S + COI).** The number in nodes represent the support values for ML (bootstrap—on top) and BI (posterior probability—below). The BI trees were not represented. The scale bar represents the average nucleotide substitutions per site. Brown arrow shows the *Capitella capitata* from type-locality (west Greenland). One asterisk (\*) indicates that only COI sequence was used and two asterisks (\*\*) indicate that only 16S sequence was used. A tilde (~) indicates that the support value was lower than 70 (ML) or 0.7 (BI), and a dash (-) indicates that the branch was not recovered. (TIF)

**S1 Table. Genetic distance.** Intraspecific (in bold) and interspecific mean pairwise genetic distances based on *p*-distance for 16S (on top) and COI (below). All values are in percentage. The number in parenthesis represents the standard error. (DOCX)

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## CAPÍTULO 3

### **A new species of *Heteromastus* (Annelida: Capitellidae) from the Brazilian coast**

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## A new species of *Heteromastus* (Annelida: Capitellidae) from the Brazilian coast

**Abstract.** A new species of *Heteromastus* has been found in samples collected on a variety of habitats on the Northeast (state of Pernambuco) and Southeast (state of São Paulo) Brazil. COI sequences were obtained and used for inter- and intraspecific comparisons. *Heteromastus* sp. n. 1. is herein described and compared to other species of the genus. The current state of knowledge on the genus is briefly given.

**Key words:** Polychaeta, morphology, molecular, cytochrome oxidase subunit 1, Brazil

### Introduction

*Heteromastus* has a thorax with 12 segments including an achaetous peristomium and 11 chaetigers, with capillary chaetae on chaetigers 1 – 5 and hooded hooks on chaetigers 6 – 11. It's a cosmopolitan genus commonly found on a variety of habitats (Hutchings and Rainer, 1981). However, only two species of this genus have been recorded from Brazil: *H. filiformis* (Claparède, 1864) and *H. similis* Southern, 1921.

Eisig (1887) created the genus *Heteromastus* by examining material from the Gulf of Genova, Italy, and reexamining specimens from other localities, such as *Capitella filiformis*, described by Claparède (1864). He concluded that *C. filiformis* had the same characteristics of *Heteromastus* and synonymized this and other species. *Capitella filiformis* was recognized by most workers as the type species of the genus, this way Hutchings and Rainer (1981) designated a neotype of *C. filiformis* in order to clarify the status of the genus *Heteromastus*. It was necessary as the type material of Claparède no longer exists and there was confusion between the generic diagnosis given by Eisig and the original description of *C. filiformis*.

Currently, there are seven valid species, *H. filiformis* (Claparède, 1864), *H. similis* Southern, 1921, *H. filobranthus* Berkeley and Berkeley, 1932, *H. giganteus* Zachs, 1933, *Heteromastus caudatus* (Hartman, 1976), first described as *Mediomastus caudatus*, *H. tohbaiensis* Yabe and Mawatari, 1998 and *H. hutchingsae* Green, 2002. Two species were described as *Heteromastus*, however, according to the original papers, the description of *H. deductus* Pillai, 1961 is related to the features of the genus *Mediomastus* (i.e., thorax with 10 chaetigers, with capillary chaetae on chaetigers 1 – 4 and hooded hooks on chaetigers 5 – 10),

and the description of *H. filiformis laminariae* Zachs, 1925 is related to the features of the genus *Parheteromastus* (*i.e.*, thorax with 11 chaetigers, with capillary chaetae on chaetigers 1 – 4 and hooded hooks on chaetigers 5 – 11). The former species has already been synonymized as *Mediomastus deductus* by Warren et al. (1994), but the latter species still needs revision.

Here we formally describe a new species on the basis of morphological as well as molecular (COI) data, *Heteromastus* sp. n. 1, and we compare it to other species of the genus (Table 1).

## Material and methods

The specimens were collected at different habitats of the Northeast (PE) and Southeast (SP) Brazil. These include a mangrove at Maracaípe, Porto de Galinhas, Pernambuco (8°S; 35°W), a mangrove and a tidal flat at Araçá bay, São Sebastião channel, São Paulo (23°48' S; 45°4' W), under the scope of the “BIOTA/FAPESP - Araçá” project, and mussel beds at Camaroeiro (23°37'S; 45°23'W) and Cidade (23°37'S; 45°23'W) beaches, Caraguatatuba bay, São Paulo, under the scope of the BIOPORE project.

A few polychaetes were fixed in formalin and preserved in 70% ethanol; however, most of the material was fixed and preserved in 92% ethanol. Specimens were examined using optical microscopy (Zeiss Axio Imager M2), stereomicroscopy (Zeiss Axio Zoom v16) and, in some cases, with a Scanning Electron Microscope (SEM). Line drawings were made with a camera lucida attached to a compound microscope and thereafter they were hand drawn with Indian ink. Measurements were made with an ocular micrometer that was calibrated with a stage micrometer. Body length was measured from the anterior margin of the prostomium to the tip of the pygidium, while the width was measured at the widest segment, excluding the chaetae. For SEM images, specimens were dehydrated in a series of ethanol solutions with progressively increasing concentrations (75 – 100%), critical-point-dried with a Balzers CPD 30 (temperature 37°C and pressure 70 kg/cm<sup>2</sup>), mounted on stubs, coated with a layer of 10–20 nm of gold, and observed under the SEM at the Laboratório de Microscopia Eletrônica, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP) using the JEOL JSM-5800 LV Scanning Electron Microscope. The material was deposited at the Museu de Zoologia, Universidade Estadual de Campinas (ZUEC), São Paulo, Brazil.

The nomenclature used for chaetal characters are:



- main fang: format and angle with the hook shaft;
- teeth: number of teeth and rows they are arranged above the main fang;
- shoulder: development of the curvature of the hook;
- hood: size, format, size of the opening and texture;
- anterior shaft: length between shoulder and node;
- node: presence or absence and shape;
- posterior shaft: length between node and the end of the hook.

Genomic DNA of specimens from the Araçá Bay was extracted with a DNeasy Blood & Tissue Kit (QIAGEN). Fragments of mitochondrial gene, cytochrome oxidase subunit 1 (COI) were amplified using degenerated primers degLCO1490 and degHCO2198 (Meyer *et al.*, 2003). PCR reactions consisted PuReTaq Ready-To-Go™ PCR Beads (GE Healthcare), 1.5 µL of each primer, 2 µL of DNA and 20 µL of water. The thermal cycling conditions for COI were one cycle of 94 °C for 3 min, 5 cycles of 94 °C for 40 s, 47 °C for 40 s and 72 °C for 1 min, 32 cycles of 94 °C for 40 s, 52 °C for 40 s and 72 °C for 1 min, followed by a final extension step of 72 °C for 5 min. PCR products were purified and sequenced by MacroGen Inc. Electropherograms were edited with Sequencher 4.1 (Gene Codes Corporation) and sequences were aligned in Mega 5.0 (Tamura *et al.*, 2011) with ClustalW tool. These new sequences were deposited in GenBank database ([www.ncbi.nlm.nih.gov/genbank](http://www.ncbi.nlm.nih.gov/genbank)).

Pairwise genetic distance was estimated using Kimura's two parameters (K2P) method (Nei & Kumar, 2000) in Mega 5.0 (Tamura *et al.*, 2011). For inter-specific comparisons sequences of *Heteromastus filiformis* were taken from GenBank (accession numbers KR916851 – KR916855).

## **Taxonomic Account**

### **Family Capitellidae Grube 1862**

#### **Genus *Heteromastus* Eisig, 1887**

Type species. *Capitella filiformis* (Claparède, 1864). Redescribed by Eisig, 1887.

Type locality. Port-Vendres, France.

**Diagnosis** (emended after Magalhães & Bailey-Brock, 2012). Prostomium *short with a palpode*, conical, sometimes tapering; eyespot present or absent. Thorax with 12 segments

including an achaetous peristomium and 11 chaetigers. Chaetiger 1 biramous. Chaetigers 1 – 5 with capillary chaetae, chaetigers 6 – 11 with long-shafted hooded hooks. Abdominal chaetigers with short-shafted hooded hooks. Branchiae may be present on posterior abdomen. *Presence of caudal cirrus.*

**Remarks.** Previously, the diagnosis of *Heteromastus* has included the characteristic of the prostomium as “short to long”, however, all described species have a short prostomium. We here maintain use the adjective “short” on the diagnosis. Additionally, all species described for the genus bear a caudal cirrus, so we include this information.

In addition, the abdominal parapodial expansions have been termed branchiae, but it is known that these structures do not have a respiratory function. At present, the only species that has such a branchiae-like structure is *Heteromastus filobranchus*.

### ***Heteromastus* sp. n. 1**

(Figures 1 – 3; Table 1)

**Holotype:** São Sebastião Channel - Araçá Bay (state of São Paulo). ZUEC POL 16745: 23°37'38"S – 45°23'50"W; intertidal soft bottom mussel bed; station 44A; 07 Oct 2006; 1 spec.

**Paratypes:** São Sebastião Channel - Araçá Bay (state of São Paulo). ZUEC POL 17661 – paratypes 1–5: 23°48'57,2"S – 45°24'29,3"W; tidal flat; station 160(3)G; 25 Jul 2013; 5 specs. ZUEC POL 17698 – paratypes 6–7: 23°48'51,4"S – 45°24'26,5"W; mangrove; station 135M; 10 Jul 2014; 2 specs. ZUEC POL 17728 – paratype 8: 23°48'51,4"S – 45°24'26,5"W; mangrove; station 53M; 19 Mar 2014; 1 spec. Caraguatatuba Bay - Camaroeiro Beach (state of São Paulo). ZUEC POL 17693 – paratype 9: 23°48'37,4"S – 45°24'21,4"W; tidal flat; station 6(1)Pr; 12 Oct 2012; 1 spec.

**Additional material examined.** São Sebastião Channel - Araçá Bay (state of São Paulo). ZUEC POL 17653: 23°48'50,2"S – 45°24'28,2"W; tidal flat; station 8(2)Pr; 12 Oct 2012; 3 specs. ZUEC POL 17654: 23°48'51,4"S – 45°24'26,5"W; mangrove; station 139M; 10 Jul 2014; 2 specs. ZUEC POL 17655: 23°48'50,2"S – 45°24'28,2"W; tidal flat; station 9(1)Pr; 12 Oct 2012; 1 spec. ZUEC POL 17656: 23°48'46,6"S – 45°24'29,8"W; mangrove; station

108M; 10 Jul 2014; 8 specs. ZUEC POL 17657: 23°49'05,1"S – 45°24'19,9"W; tidal flat; station 17(1)Pr; 13 Oct 2012; 1 spec. ZUEC POL 17658: 23°48'39,2"S – 45°24'17,5"W; tidal flat; station 11(4)Pr; 13 Oct 2012; 2 specs. ZUEC POL 17659: 23°48'51,4"S – 45°24'26,5"W; tidal flat; station 172(1)G; 16 Sep 2013; 4 specs. ZUEC POL 17660: 23°48'57,2"S – 45°24'29,3"W; tidal flat; station 160(3)G; 25 Jun 2013; 40 specs. ZUEC POL 17662: 23°48'39,4"S – 45°24'26,2"W; tidal flat; station 114(2)Pr; 17 Sep 2013; 1 spec. ZUEC POL 17663: 23°48'50,2"S – 45°24'28,2"W; tidal flat; station 82(2)Pr; 24 Jun 2013; 4 specs. ZUEC POL 17664: 23°48'39,2"S – 45°24'17,5"W; tidal flat; station 122(1)Pr; 17 Sep 2013; 1 spec. ZUEC POL 17665: 23°48'54,4"S – 45°24'26,6"W; tidal flat; station 86(2)Pr; 24 Jun 2013; 3 specs. ZUEC POL 17666: 23°48'54,4"S – 45°24'26,6"W; tidal flat; station 123(3)Pr; 16 Sep 2013; 10 specs. ZUEC POL 17667: 23°48'37,4"S – 45°24'21,4"W; tidal flat; station 117(2)Pr; 17 Sep 2013; 1 spec. ZUEC POL 17668: 23°48'45,8"S – 45°24'28,9"W; tidal flat; station 142(3)G; 14 Oct 2012; 35 specs. ZUEC POL 17669: 23°48'54,4"S – 45°24'26,5"W; mangrove; station 77M; 10 Jul 2014; 45 specs. ZUEC POL 17670: 23°48'54,4"S – 45°24'26,5"W; mangrove; station 81M; 10 Jul 2014; 48 specs. ZUEC POL 17671: 23°48'54,4"S – 45°24'26,5"W; mangrove; station 11M; 17 Mar 2014; 43 specs. ZUEC POL 17672: 23°48'46,6"S – 45°24'29,8"W; mangrove; station 45M; 18 Mar 2014; 6 specs. ZUEC POL 17673: 23°48'46,3"S – 45°24'25,2"W; tidal flat; station 10(1)Pr; 12 Oct 2012; 1 spec. ZUEC POL 17674: 23°48'40,1"S – 45°24'23,1"W; tidal flat; station 79(4)Pr; 24 Jun 2013; 1 spec. ZUEC POL 17675: 23°48'51,4"S – 45°24'26,5"W; tidal flat; station 8(2)Pr; 12 Oct 2012; 7 specs. ZUEC POL 17676: 23°48'46,3"S – 45°24'25,2"W; tidal flat; station 10(3)Pr; 12 Oct 2012; 1 spec. ZUEC POL 17677: 23°48'39,4"S – 45°24'26,2"W; tidal flat; station 40(3)Pr; 24 Feb 2013; 1 spec. ZUEC POL 17678: mangrove; 17 Mar 2015; 1 spec. ZUEC POL 17679: 23°48'57,2"S – 45°24'29,3"W; tidal flat; station 75(3)A; 09 May 2012; 5 specs. ZUEC POL 17680: 23°48'57,5"S – 45°24'28,5"W; tidal flat; station 108(2)A; 30 Jul 2012; 3 specs. ZUEC POL 17681: 23°48'54,4"S – 45°24'26,6"W; tidal flat; station 12(4)Pr; 12 Oct 2012; 4 specs. ZUEC POL 17682: 23°48'51,4"S – 45°24'26,5"W; tidal flat; station 8(3)Pr; 12 Oct 2012; 1 spec. ZUEC POL 17683: 23°48'51,4"S – 45°24'26,5"W; mangrove; station 126M; 10 Jul 2014; 1 spec. ZUEC POL 17684: 23°48'54,4"S – 45°24'26,5"W; mangrove; station 21M; 17 Mar 2014; 17 specs. ZUEC POL 17685: 23°48'54,4"S – 45°24'26,5"W; mangrove; station 9M; 17 Mar 2014; 16 specs. ZUEC POL 17686: 23°48'54,4"S – 45°24'26,5"W; mangrove; station 20M; 17 Mar 2014; 29 specs. ZUEC POL 17687: 23°48'54,4"S – 45°24'26,5"W; mangrove; station 8M; 17 Mar 2014; 1 spec. ZUEC POL

17688: 23°48'45,8"S – 45°24'28,9"W; tidal flat; station 162(1)G; 24 Jun 2013; 4 specs. ZUEC POL 17689: 23°48'51,4"S – 45°24'26,5"W; mangrove; station 66M; 19 Mar 2014; 9 specs. ZUEC POL 17690: 23°48'37,4"S – 45°24'21,4"W; tidal flat; station 80(4)Pr; 24 Jun 2013; 1 spec. ZUEC POL 17691: 23°48'47,3"S – 45°24'20,4"W; tidal flat; station 14(3)Pr; 13 Oct 2012; 1 spec. ZUEC POL 17692: 23°48'54,4"S – 45°24'26,5"W; mangrove; station 13M; 17 Mar 2014; 11 specs. ZUEC POL 17694: 23°48'37,4"S – 45°24'21,4"W; tidal flat; station 6(1)Pr; 12 Oct 2012; 4 specs. ZUEC POL 17695: 23°48'55,3"S – 45°24'31,5"W; tidal flat; station 118(3)Pr; 16 Sep 2013; 1 spec. ZUEC POL 17696: 23°48'54,4"S – 45°24'26,5"W; mangrove; station 2M; 17 Mar 2014; 21 specs. ZUEC POL 17697: 23°48'51,4"S – 45°24'26,5"W; mangrove; station 135M; 10 Jul 2014; 7 specs. ZUEC POL 17699: 23°48'57,2"S – 45°24'29,3"W; tidal flat; station 140(1)G; 14 Oct 2012; 4 specs. ZUEC POL 17700: 23°48'54,4"S – 45°24'26,5"W; mangrove; station 78M; 10 Jul 2014; 8 specs. ZUEC POL 17701: 23°48'39,4"S – 45°24'26,2"W; tidal flat; station 40(2)Pr; 24 Feb 2013; 2 specs. ZUEC POL 17702: 23°48'39,4"S – 45°24'26,2"W; tidal flat; station 3(4)Pr; 12 Oct 2012; 1 spec. ZUEC POL 17703: 23°48'46,6"S – 45°24'29,8"W; mangrove; station 44M; 18 Mar 2014; 1 spec. ZUEC POL 17704: 23°48'54,4"S – 45°24'26,5"W; mangrove; station 75M; 10 Jul 2014; 50 specs. ZUEC POL 17705: 23°48'51,4"S – 45°24'26,5"W; mangrove; station 137M; 10 Jul 2014; 3 specs. ZUEC POL 17706: 23°48'51,4"S – 45°24'26,5"W; mangrove; station 140M; 10 Jul 2014; 4 specs. ZUEC POL 17707: 23°48'54,4"S – 45°24'26,5"W; mangrove; station 82M; 10 Jul 2014; 52 specs. ZUEC POL 17708: 23°48'43,9"S – 45°24'26,4"W; tidal flat; station 115(1)Pr; 17 Sep 2013; 2 specs. ZUEC POL 17709: 23°48'50,2"S – 45°24'28,2"W; tidal flat; station 45(2)Port; 23 Feb 2013; 3 specs. ZUEC POL 17710: 23°48'51,4"S – 45°24'26,5"W; mangrove; station 129M; 10 Jul 2014; 3 specs. ZUEC POL 17711: 23°48'51,4"S – 45°24'26,5"W; mangrove; station 65M; 19 Mar 2014; 18 specs. ZUEC POL 17712: 23°48'54,4"S – 45°24'26,5"W; mangrove; station 89M; 10 Jul 2014; 33 specs. ZUEC POL 17724: 23°48'54,4"S – 45°24'26,5"W; mangrove; station 12M; 17 Mar 2014; 11 specs. ZUEC POL 17725: 23°48'54,4"S – 45°24'26,5"W; mangrove; station 3M; 18 Mar 2014; 18 specs. ZUEC POL 17726: 23°48'54,4"S – 45°24'26,5"W; mangrove; station 12M; 17 Mar 2014; 15 specs. ZUEC POL 17727: 23°48'46,6"S – 45°24'29,8"W; mangrove; station 35M; 18 Mar 2014; 6 specs. **Caraguatatuba Bay - Camaroeiro Beach (state of São Paulo).** ZUEC POL 16763: 23°37'38"S – 45°23'50"W; intertidal soft bottom mussel bed; station 48A; 02 Nov 2006; 1 spec. ZUEC POL 17714: 23°37'38"S – 45°23'50"W; intertidal soft bottom mussel bed; station 16A; 25 May 2006; 1 spec. ZUEC POL 17717: 23°37'38"S –

45°23'50"W; intertidal soft bottom mussel bed; station 24A; 23 Apr 2006; 1 spec. ZUEC POL 17718: 23°37'38"S – 45°23'50"W; intertidal soft bottom mussel bed; station 38A; 23 Apr 2006; 1 spec. ZUEC POL 17719: 23°37'38"S – 45°23'50"W; intertidal soft bottom mussel bed; station 59A; 04 Jan 2007; 1 spec. ZUEC POL 17720: 23°37'38"S – 45°23'50"W; intertidal soft bottom mussel bed; station 53A; 04 Dec 2006; 1 spec. ZUEC POL 17721: 23°37'38"S – 45°23'50"W; intertidal soft bottom mussel bed; station 28A; 11 Jul 2006; 1 spec. ZUEC POL 17722: 23°37'38"S – 45°23'50"W; intertidal soft bottom mussel bed; station 61A; 05 Nov 2006; 2 specs. ZUEC POL 17723: 23°37'38"S – 45°23'50"W; intertidal soft bottom mussel bed; station 65A; 05 Feb 2007; 1 spec. **Caraguatatuba Bay - Cidade Beach (state of São Paulo)**. ZUEC POL 17713: 23°37'27"S – 45°23'56"W; intertidal soft bottom mussel bed; station 39V; 05 Jan 2007; 1 spec. ZUEC POL 17715: 23°37'27"S – 45°23'56"W; intertidal soft bottom mussel bed; station 3V; 28 Apr 2006; 1 spec. ZUEC POL 17716: 23°37'27"S – 45°23'56"W; intertidal soft bottom mussel bed; station 21V; 08 Sep 2006; 1 spec. **Porto de Galinhas - Maracaípe mangrove (state of Pernambuco)**. ZUEC POL 20570: 8.31°S – 35°W; mangrove; 20 May 2015; 37 specs.

**Description.** Size range of complete individuals 5.5 – 47 mm long, 0.3 – 1.0 mm wide for 33 – 145 chaetigers. Body elongate, slender, slightly widest anteriorly, gradual narrowing posteriorly. Color red in life; pale yellow in alcohol. Prostomium conical, small with a terminal palpode (Figs. 1A–B, 2A–C and 3A, C). Nuchal organs filamentous located at basis of the prostomium (Figs. 2D and 3B). Two black subdermal eyespots (Fig. 2B). Proboscis globular-shaped, with several robust papillae (Figs. 1A–B and 3C). Peristomium achaetous, as long as thoracic chaetigers (Fig. 3A, C). Thorax with 11 chaetigers, slightly widest anteriorly gradually narrowing to chaetiger 11 (Figs. 1A–B, 2A and 3A). Peristomium and first four chaetigers with slightly rugose cuticle (Figs. 1B and 2A–C). First chaetiger biramous. Thoracic chaetigers weakly biannulate, with a lateral groove (Figs. 2A and 3A). Adult specimens with bilimbate capillaries (Figs. 2G and 3E) in notopodia and neuropodia of chaetigers 1 – 5, replaced by hooded hooks in notopodia and neuropodia of chaetigers 6 – 11. Noto- and neurosetae arranged in a single row of 5 – 14 and 4 – 16 capillaries, respectively, and 3 – 10 hooded hooks. Right and left dorsal bundles of chaetae widely separated on chaetiger 1, reducing the interval up to the last thoracic chaetigers. Lateral organs between noto- and neuropodia, in the middle of chaetigers 1 – 11 (Figs. 1B, 2E and 3A, D); indistinct on abdomen. Four pairs of intersegmental genital pores (in line, but posterior to lateral



organs) between chaetigers 1/2, 2/3, 3/4, 4/5, 5/6, 6/7, 7/8, 8/9 and 9/10 (Fig. 3A, C); indistinct in smaller specimens. Thoracic hooded hooks 232 – 343  $\mu\text{m}$  length; short and pointed main fang at right angle with shaft, protruding just slightly through frontal opening, surmounted by 3 – 4 apical teeth arranged in two rows (2 – 3 basally and one apically); indistinct shoulder; anterior shaft absent; node absent; long and straight shaft; long and smooth hood (Figs. 2H, I and 3K, L). Transition between thorax and abdomen indistinct (Figs. 1A, 2A and 3A). Abdominal chaetigers as long as wide (Fig. 2A). Anterior right and left notopodial bundles dorsal, close to each other; bundles dorso-lateral on middle and posterior abdominal chaetigers. Notopodial lobes absent on anterior chaetigers, poorly developed semi-triangular expansions on middle abdomen and well-developed after chaetiger 48 (Figs. 1A, F, 2A, F and 3F, H, I). Anterior and middle abdomen with poorly developed neuropodial lobes and posterior abdomen with expanded lobes (Figs. 2F and 3F, G). Chaetigers with 7 – 16 hooded hooks in notopodia and 9 – 17 in neuropodia, reduced to two hooks in posterior abdomen. Abdominal hooded hooks 81.25 – 203  $\mu\text{m}$  long, smaller than thoracic hooks. Notopodial hooks with pointed and large main fang at right angle with shaft, surmounted by eight teeth arranged in three rows (3 basally, 3 median and 2 apically) with a straight aperture of hood (Fig. 3M). Neuropodial hooks with pointed and large main fang at right angle with shaft, surmounted by 13 teeth arranged in four rows (2–5–4–2) with a larger aperture of hood; short curved shoulder; short anterior shaft; well-developed node; long and curved posterior shaft; short and smooth hood (Figs. 2J, K and 3N, O). Branchiae absent. Pygidium with short, digitate mid-ventral caudal cirrus; with terminal anal aperture (Figs. 1G, 2F and 3J).

**Methyl green staining pattern.** Chaetigers 1 – 6 darkly stained; abdomen uniformly stained.

**Variation.** Specimens from Araçá Bay ranged from 5.5 – 47 mm long, 0.3 – 1.0 mm wide and 33 – 145 chaetigers with notopodial expansion beginning from chaetigers 48 and 56. Specimens from Camaroeiro beach ranged from 0.3 – 0.95 mm wide with notopodial expansions beginning from chaetiger 48; only complete specimen with 32 mm long and 90 chaetigers. Specimens from Cidade beach ranged from 0.5 – 0.7 mm wide with notopodial expansions beginning from chaetigers 42 and 48. Specimens from Maracaípe mangrove ranged from 9.5 – 26 mm long, 0.45 – 1.0 mm wide and 46 – 96 chaetigers with notopodial expansions beginning from chaetigers 48 and 56.

**Biology.** Mature oocytes were observed in the coelom of 20% of specimens wider than 0.5 mm from Araçá bay and ranged from 200 – 250  $\mu\text{m}$ . Mature oocytes were observed in the coelom of 32% of specimens wider than 0.7 mm from Maracaípe mangrove and ranged from 150 – 250  $\mu\text{m}$ . Mature oocytes were not observed in specimens from the Camaroeiro and Cidade beaches.

**Remarks.** *Heteromastus filobranthus* and *H. caudatus* are the most different species from *Heteromastus* sp. n. 1 by the absence of eyespots, hooded hooks with two rows of teeth and a pygidium with very slender, small cirrus. Additionally, *H. filobranthus* has filiform branchiae and *H. caudatus* has abdominal chaetigers much longer than wide with long posterior hooded hooks projecting from the body wall, resembling spines.

In addition, *Heteromastus filiformis* has notopodial lobes as semi-triangular expansions and hooded hooks with several teeth arranged in three rows; this species differs from *Heteromastus* sp. n. 1 by the absence of eyespots, distinct demarcation between thorax and abdomen, intersegmental genital pores starting between chaetigers 6/7, and abdominal hooded hooks at least three times longer (0.078 mm) than the thoracic hooks (0.022 mm). The *H. filiformis* specimens examined by Magalhães & Bailey-Brock (2012) from the Oahu Island also lacked eyespots, the abdominal hooded hooks were longer than the thoracic hooks, and neither branchiae or notopodial expansions were observed.

*Heteromastus* sp. n. 1 resembles *H. tohbaiensis* by having subdermal eyespots, a small conical prostomium, the shape and number of teeth of abdominal hooded hooks which are smaller than the thoracic hooks, and shape of the pygidium and caudal cirrus. However, both species can be distinguished by the following characteristics on *H. tohbaiensis*: epithelium of thoracic segments smooth; lateral organs starting on the fourth chaetiger; thoracic hooded hooks with a distinct node and straight teeth; distinct transition between thorax and abdomen; and absence of notopodial lobes as semi-triangular expansions. The figure presented by the authors show equal expansion for both noto- and neuropodial lobes (Yabe & Mawatari, 1998).

Although *Heteromastus hutchingsae* also has the peristomium and first thoracic chaetigers slightly rugose, lateral organs on the entire thorax, anterior and middle abdomen with reduced neuropodial lobes, notopodial lobes as semi-triangular expansions, and thoracic hooded hooks longer than the abdominal hooks (Green, 2002), this species differs from *Heteromastus* sp. n. 1 by the absence of eyespots, presence of intersegmental genital pores

starting between chaetigers 7/8, pygidium with two fused cirrus, and several teeth on thoracic hooded hooks.

Finally, *Heteromastus similis* differs from *Heteromastus* sp. n. 1 by the absence of eyespots, first and second chaetigers with dorsal capillaries arranged in two rows, change in shape between the anterior and posterior abdominal chaetigers, and expanded neuropodial lobes beginning posteriorly to notopodial expansions. Specimens of *H. similis* examined by Gallardo (1967) from South Viet Nam and India also differ from the new species by the absence of eyespots and presence of a short peristomium.

**Habitat.** Intertidal region, in tidal flat, mangrove and mussel beds.

**Type locality.** Aracá Bay, São Paulo, Brazil (South Atlantic Ocean).

**Distribution.** South Atlantic Ocean: Brazil (states of Pernambuco and São Paulo).

**Molecular identity.** COI fragments of 658 bases pair (bp) were obtained from three individuals, from Araçá bay, São Paulo. The COI sequences were deposited in GenBank under the accession numbers, KY427885- KY427887. The intra-specific genetic distance varied from 0.3% – 0.6% (p-distance and K2P). The inter-specific genetic distance between *H. filiformis* (unique species of genus found on GenBank) and *Heteromastus* sp. n. 1 was 36.7% – 37.4% (p-distance - K2P).

## Acknowledgments

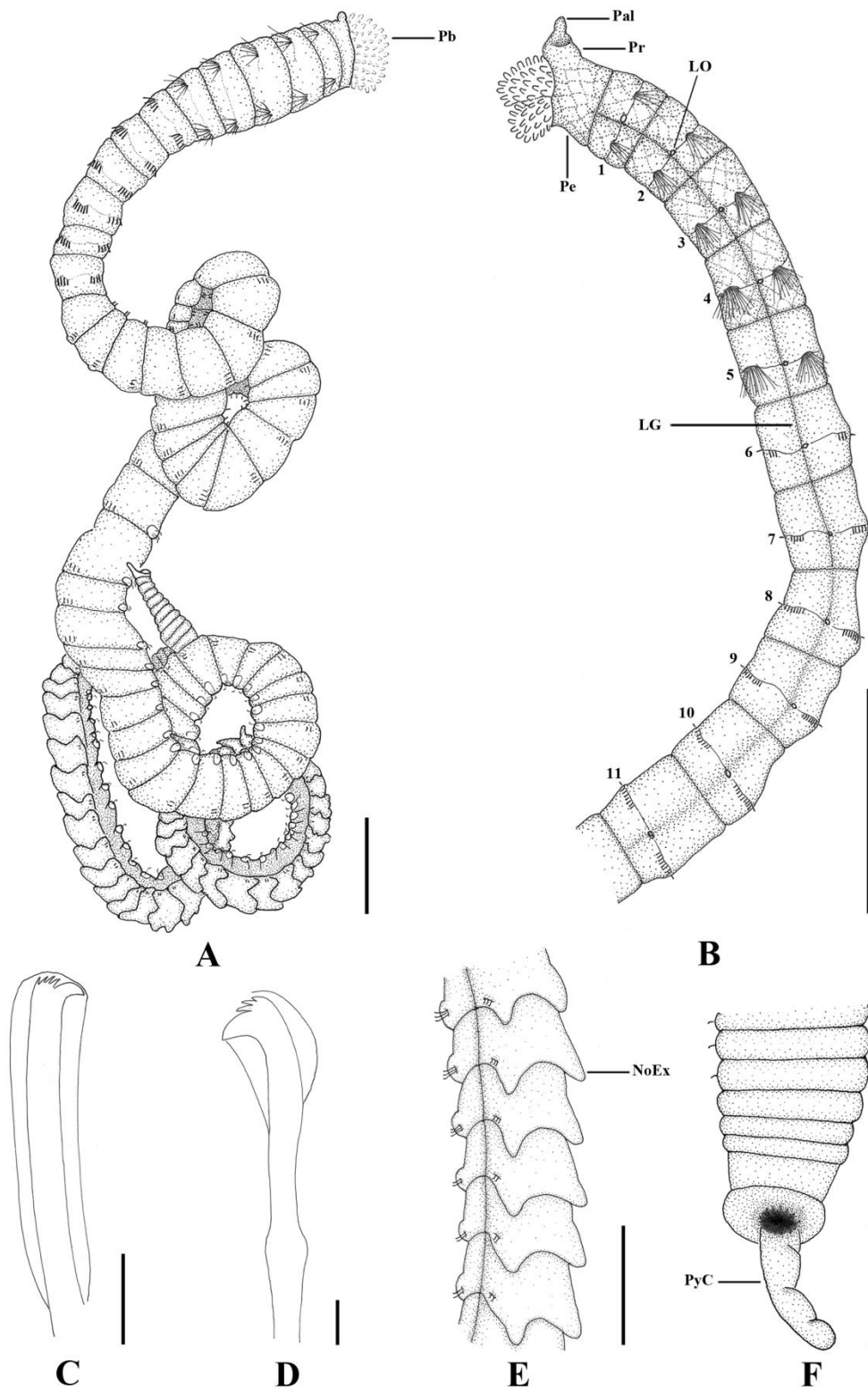
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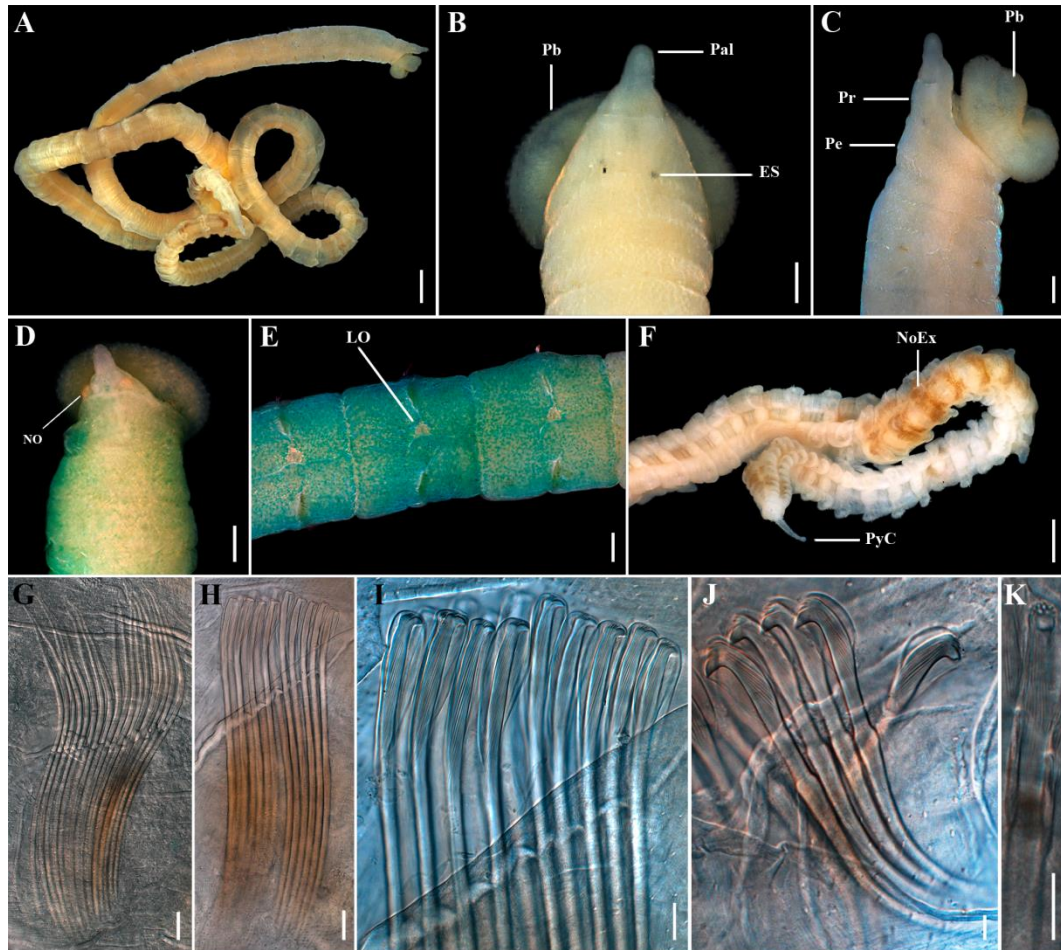
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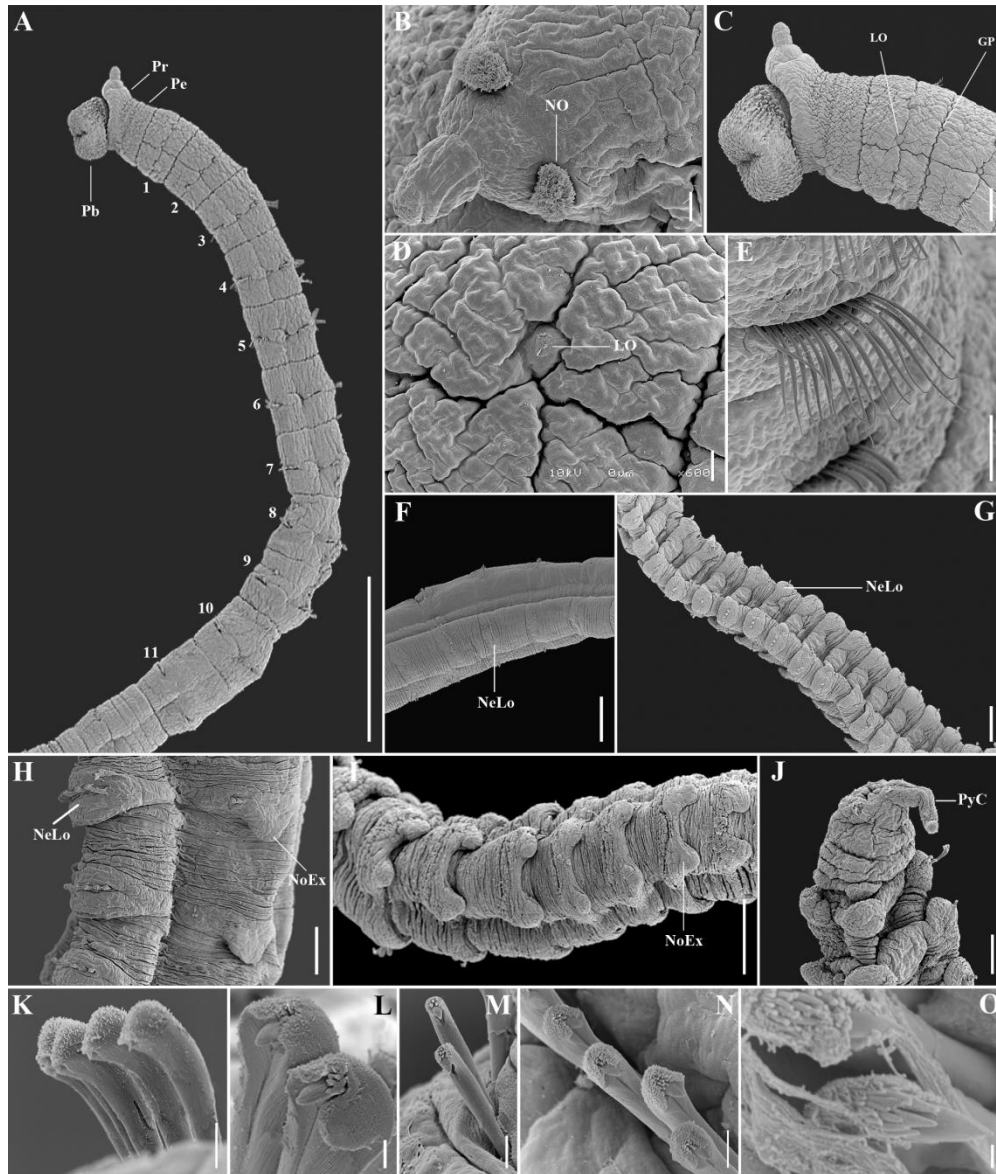




**Fig. 1.** *Heteromastus* 1 sp. n. (A) Complete specimen; (B) Thorax, lateral view; (C) Thoracic hooded hook, lateral view; (D) Abdominal hooded hook; lateral view; (E) Abdominal region, dorso-lateral view; (F) Pygidium, ventral view. Pal: palpode. Pb: propodeis. Pr: prostomium. LO: lateral organ. LG: lateral groove. NoEx: notopodial expansions. PyC: pygidial cirri. Scale bars: A, B, 1 mm; C, D, 10 µm; E, F, 0.5 mm.



**Fig. 2.** *Heteromastus* 1 sp. n. (A) Complete specimen; (B) Anterior end, dorsal view; (C) Anterior end, lateral view; (D) Nuchal organ; (E) Lateral organ; (F) Posterior end and pygidium; (G) Capillary chaetae; (H) Thoracic hooded hooks, entire; (I) Thoracic hooded hooks, lateral view; (J) Abdominal hooded hook, lateral view; (K) Abdominal hooded hook, frontal view. Pal: palpo. Pb: propocis. ES: eyespots. Pe: peristomium. Pr: prostomium. NO: nuchal organ. LO: lateral organ. NoEx: notopodial expansions. PyC: pygidial cirrus. Scale bars: A, 0.5 mm; B, C, D, E, 0.1 mm; F, 0.2 mm; G, H, 20 µm; I, J, K, 10 µm.



**Fig. 3.** *Heteromastus* 1 sp. n., SEM. (A) Thoracic region, lateral view; (B) Nuchal organs; (C) Anterior end, lateral view; (D) Lateral organ; (E) Capillary chaetae; (F) Anterior abdominal region; (G) Posterior abdominal region; (H) Anterior parapodial expansion; (I) Posterior parapodial expansion; (J) Posterior end and pygidium; (K) Thoracic hooded hooks, lateral view; (L) Thoracic hooded hooks, frontal view; (M) Notopodial abdominal hooded hook, frontal view; (N) Neuropodial abdominal hooded hook, frontal view; (O) Neuropodial abdominal hooded hook, teeth detail. Pb: proposcis. Pe: peristomium. Pr: prostomium. NO: nuchal organ. LO: lateral organ. GP: genital pore. NeLo: neuropodial lobes. NoEx: notopodial expansions. PyC: pygidial cirrus. Scale bars: A, 1 mm; B, D, 20  $\mu$ m; C, G, I, 0.1 mm; E, H, J, 0.05 mm; F, 0.2 mm; K, M, N, 5  $\mu$ m; L, 2  $\mu$ m; O, 1  $\mu$ m.

**Table 1.** Comparison among *Heteromastus* species. L: length; W: width.

| Species  | Eyes   | Notopodial expansions                                 | Capillaries chaeta | Thoracic hooks | Abdominal hooks  | Pigidium              | Maximum size (L x W) mm | N° segments | Type locality                        | Habitat                             | References                |
|--|--------|---|--------------------|----------------|--|-----------------------|-------------------------|-------------|--------------------------------------|-------------------------------------|---------------------------|
| <i>Heteromastus caudatus</i> (Hartman, 1976)               | Absent | Absent  | —                  | —              | —  | single ventral cirrus | 20 x 0.7                | 50          | Bay of Bengal and Arabian Sea        | estuaries and shallow subtidal; mud | Hartman, 1976             |
| <i>Heteromastus filiformis</i> (Claparède, 1864)           | Absent | posterior triangular expansions reaching next setiger | 12–18              | —              | 8–9 noto and 10–14 neuro; 11–15 teeth above main fang; with developed node | single ventral cirrus | 40 x 0.6                | 230         | Port-Vendres, France                 |                                     | Hutchings & Reiner, 1981  |
| <i>Heteromastus filibranchus</i> Berkeley & Berkeley, 1932 | Absent | Twelve to sixteen filiform threads                    | —                  |                |  | single ventral cirrus | 150 x 2                 | 250         | Nanoose Bay, Vancouver               | subtidal area (15 – 20 m)           | Berkeley & Berkeley, 1932 |
| <i>Heteromastus giganteus</i> Zachs, 1933                  | Absent | —   | —                  | —              | —  | —                     | 5 x 0.25                | —           | Pacific Ocean, northern Sea of Japan | —                                   | Zachs, 1933               |

Table 1. (Continued).

| Species   | Eyes    | Notopodial expansions   | Capillaries chaeta   | Thoracic hooks   | Abdominal hooks  | Pigidium                 | Maximum size (L x W) mm | Nº segments | Type locality                                 | Habitat  | References            |
|---|---------|---|----------------------|--|--|--------------------------|-------------------------|-------------|---|--|-----------------------|
| <i>Heteromastus hutchingsae</i> Green, 2002           | Absent  | posterior triangular expansions reaching next setiger                                   | —                    | several teeth above main fang; long shaft; with slightly inflated node | 7–8 teeth above main fang in 3 rows; short shaft; with well developed node                     | two fused ventral cirrus | — x 0.6                 | 99          | Andaman Sea, South China                      | mud and muddy sand; subtidal area (20 – 61 m)            | Green, 2002           |
| <i>Heteromastus tohbaiensis</i> Yabe & Mawatari, 1998 | Present | posterior parapodial lobes slightly expanded  | 5–10 noto and neuro  | 3–6 per ramus; ~30 straight teeth above main fang in 3–4 rows; with    | 3–6 per ramus; ~10 curved teeth above main fang in 2–3 rows; with 10–15 per ramus; two rows of | single ventral cirrus    | 21.5 x 0.4              | 130         | Akkeshi Lake, Japan                           | fine mud; subtidal area                                  | Yabe & Mawatari, 1998 |
| <i>Heteromastus similis</i> Southern, 1921            | Absent  | posterior triangular expansions reaching next setiger (after 100 <sup>th</sup> setiger) | 7–15 noto 4–14 neuro | two teeth above main fang; long shaft; without node                    | teeth in lateral view; short shaft; with well developed node                                   | single ventral cirrus    | 55 x —                  | 212         | Chilka Lake, India                            | intertidal and shallow subtidal area                     | Southern, 1921        |
| <i>Heteromastus</i> 1 sp. n.                          | Present | posterior triangular expansions reaching next setiger                                   | 5–14 noto 4–16 neuro | 3–4 teeth above main fang in two rows; long shaft; without node        | noto: 8 teeth in 3 rows; neuro: 12 teeth in 3 rows; short shaft; with well developed node      | single ventral cirrus    | 33 x 0.5                | 125         | South Atlantic Ocean: São Paulo State, Brazil | intertidal area, in tidal flat, mangrove and mussel beds | Current paper         |



## CAPÍTULO 4

**Species of *Rashgua* Wesenberg-Lund, 1949 (Annelida:**

**Capitellidae) from Southeast Brazil**

## Species of *Rashgua* Wesenberg-Lund, 1949 (Annelida: Capitellidae) from Southeast Brazil

**Abstract.** Specimens of the genus *Rashgua* have been misinterpreted as *Notomastus* for a long time because they have the same number of thoracic chaetigers. Although thoracic formula has been the main character for distinguishing Capitellidae genera, it's an unsatisfactory feature since it may change with age and sexual maturity; besides, chaetal distribution is variable even among species of the same genus. *Rashgua* has 11 thoracic chaetigers as many other genera, but it is unique in lacking the majority of the notopodial chaetae of the abdomen. Three species of *Rashgua* have been found on samples collected on a variety of depths of the Southeast Brazil. Two new species are herein described and compared to other species of the genus, however, one of them has only a single incomplete specimens. This way, it wasn't referred as a new species, until new specimens have been sorted, but it was included due to the importance of the new record to Brazilian waters. The current state of knowledge of the genus is given, and a comparative table and a taxonomic key as well.

**Key words:** Polychaeta, morphology, intertidal region, Continental Shelf, Slope, Brazilian coast.

### Introduction

*Rashgua* is unique in lacking the majority of the notopodial chaetae of the abdomen. This genus was originally created by Wesenberg-Lund (1949) with the description of *R. rubrocincta*, a species with eleven thoracic chaetigers having only capillaries and abdominal segments entirely lacking notopodial chaetae. However, Hartman (1947) described a species, *Notomastus lobatus*, also lacking abdominal notopodial chaetae and placed it within *Notomastus* due to its thoracic chaetal formula. Later, Ewing (1982) considered *Rashgua* as synonym of *Notomastus* because both genera had many features in common.

The genus *Rashgua* had not been reported since its original description until Green (2002) described an additional species, *R. branchiatus*. The author redefined the genus and considered it distinct from *Notomastus* not only based on the absence of notopodial chaetae in most abdominal segments but also in having small dorsolateral lobes on the abdomen, protruded lateral organs and enlarged neuropodial lobes. Green (2002) reassigned *N. lobatus*

to the genus *Rashgua* and expanded the diagnosis of the latter genus to include the presence of notopodial chaetae in the first two abdominal segments on the new species.

Correct identification of Capitellidae specimens is very difficult mainly because there are few distinctive characters at the generic and specific levels and the definition of genera is rather controversial. Thoracic formula has been the main character for distinguishing genera, however, it is recognized that this feature is unsatisfactory (Hartman, 1947; Fauchald, 1977; Rouse and Pleijel, 2001; Green, 2002). Chaetal distribution, particularly on posterior thorax, may change with age and sexual maturity, furthermore, juveniles may have fewer thoracic chaetigers than adults (Ewing, 1982, 1984; Blake, 2000).

Beyond the fact that some genera have species with a range of thoracic chaetigers, such as *Mediomastus* and *Scyphoproctus*, which makes the identification more difficult, another point that also becomes a problem to identify capitellids is that several genera show similar or identical thoracic arrangement. This was the reason specimens of the genus *Rashgua* have been misinterpreted as *Notomastus* for a long time, since Green (2002) included more characters to the diagnosis of the former genus.

Currently, there are three valid species, *Rashgua rubrocincta* Wesenberg-Lund, 1949, *R. lobatus* (Hartman, 1947) and *R. branchiatus* Green, 2002. To date, only one species of this genus has been recorded for the Brazilian coast, *R. lobatus*, this way, the aim of this study was to investigate the diversity of the genus of the Southeast Brazil. Here, we describe a new species, and a taxonomic key and a comparative table are given (Table 1).

## Material and Methods

The specimens were collected at different habitats of the Southeast Brazil, under the scope of three independent projects:

- 1) HABITATS/CENPES/PETROBRAS – “Campos Basin Environmental Heterogeneity” (2008 – 2009) (21° – 24°S / 38° – 45°W), with focus on soft bottoms of four habitats (12 – 3301 m), the Paraíba do Sul river mouth, the Continental Shelf, the Slope, and the canyons Almirante Câmara (CANAC) and Grussaí (CANG), of the Campos Basin, states of Espírito Santo and Rio de Janeiro, Southeast Brazil (for details see Lavrado and Brasil, 2010);
- 2) AMBES – “Environmental Characterization of the Espírito Santo Basin” (2010 – 2013) (18° – 21°S / 37° – 40°W), with focus on soft bottoms of four habitats (25 –

3000 m), the Rio Doce river mouth, the Continental Shelf, the Slope, and the canyons Watu Norte (CANWN) and Rio Doce (CAND) of the Espírito Santo Basin, state of Espírito Santo, Southeast Brazil;

- 3) BIOTA/FAPESP - Araçá “Biodiversity and functioning of a subtropical coastal ecosystem: subsidies for integrated management” (2012 – 2016) (23°48'S – 45°4'W), the aim of this project was to study the fauna of different habitats (0 – 25 m): mangrove, tidal and subtidal region of the Araçá Bay, São Sebastião Channel, northern coast of the state of São Paulo, Southeast Brazil (for details see Amaral *et al.*, 2016).

Specimens were examined using optical microscopy (Zeiss Axio Imager M2), stereomicroscopy (Zeiss Axio Zoom v16) and, in some cases, with a Scanning Electron Microscope (SEM). Line drawings were made with a camera lucida attached to a compound microscope and afterwards hand drawn with Indian ink. Measurements were taken with an ocular micrometer that was calibrated with a stage micrometer. The body length was measured from the anterior margin of the prostomium to the tip of the pygidium, while the width was measured at the widest segment, excluding the chaetae. For SEM images, specimens were dehydrated in a series of ethanol solutions with progressively increasing concentrations (75 – 100%), critical-point-dried with a Balzers CPD 30 (temperature 37 °C and pressure 70 kg/cm<sup>2</sup>), mounted on stubs, covered with a layer of 10 – 20 nm of gold, and observed under the SEM at the Laboratório de Microscopia Eletrônica, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP) using the JEOL JSM-5800 LV Scanning Electron Microscope.

The nomenclature used for chaetal morphology follows the usually used for Capitellidae species and some suggested by Green (2002). The chaetal characters used here are:

- main fang: format and angle with the hook shaft;
- teeth: number of teeth and rows they are arranged above the main fang;
- shoulder: development of the curvature of the hook;
- hood: size, format, size of the opening and texture;
- anterior shaft: length between shoulder and node;
- node: presence or absence and shape;
- posterior shaft: length between node and the end of the hook.

The material was deposited at the Museu de Zoologia, Universidade Estadual de Campinas (ZUEC).

## **Taxonomic Account**

### **Family Capitellidae Grube, 1862**

### **Genus *Rashgua* Wesenberg-Lund, 1949**

Type species. *Rashgua rubrocincta* Wesenberg-Lund, 1949

Type locality. Persian Gulf.

**Diagnosis** (emended after Green, 2002). Thorax *strongly tessellated* with 12 segments including an achaetous peristomium and 11 chaetigers with capillary chaeta. *Eyespots absent or present*. Abdomen with neuropodial hooded hooks; *notochaetae absent or present on the first few chaetigers*. Abdomen with enlarged neuropodial lobes, *with or without dorsolateral expansions; with or without* small notopodial lobes; and protruded lateral organs. Hooded hooks long-shafted with bulbous node and *usually one or two rows of teeth above the main fang*. Branchia *absent*.

**Remarks:** The diagnosis was expanded to accommodate variations of the characters or to include important characters to the genus. For example, all species have strongly tessellated epithelium, besides different patterns, which appears to be a significant feature of the group. *Rashgua branchiatus* bears notopodial hooded hooks just on the first two abdominal chaetigers, so we judge helpful to discriminate the position this character may have. Additionally, the presence of branchiae was excluded since it is known that these structures do not have respiratory functions because capitellids lack a circulatory system and these projections from the body wall contain coelomic extensions rather than loops from the circulatory system (Fauchald and Rouse, 1997). Here, these structures are called “neuropodial expansions”. Finally, the generic description is emended to accommodate the differences of the species *Rashgua* sp. B, which has eyespots, a new character for the genus, but it lacks both small notopodial lobes and dorsolateral expansions of the neuropodial lobe.

### **Key to all valid species of *Rashgua***

- 1a.** Presence of eyespots; absence of small notopodial lobes and neuropodial expansions; hooded hooks with 8 – 9 teeth arranged in a single row.....*Rashgua* sp. **B**
- 1b.** Absence of eyespots; presence of small notopodial lobes (Fig. 5B) and neuropodial expansions of the lobe (Fig. 2G).....**2**
- 2a.** Neuropodial expansions globulous.....**3**
- 2b.** Neuropodial expansions digitated.....**4**
- 3a.** Neuropodial expansions throughout the abdomen; notopodial lobes reduced, throughout the abdomen; one row above main fang.....*Rashgua rubrocincta*
- 3b.** Neuropodial expansions throughout the abdomen; notopodial lobes triangular, throughout the abdomen; hooded hooks with 4 teeth arranged in a single row..... *Rashgua* **A sp. n.**
- 4a.** Neuropodial expansions on anterior abdomen, between chaetiger 15 and 33; notopodial lobes reduced, throughout the abdomen; hooded hooks with 5 teeth arranged in two rows; hooded hooks present on first two abdominal chaetigers.....*Rashgua branchiatus*
- 4b.** Neuropodial expansions on anterior abdomen, between chaetigers 13 and 23; hooded hooks with 4 – 5 teeth arranged in a single row.....*Rasgua lobatus*

***Rashgua lobatus* (Hartman, 1947)**

(Figures 1 – 3; Table 1)

*Notomastus lobatus* Hartman, 1947: 415 – 417, pl. 51, figs. 1 – 5; 1969: 399, figs. 1 – 5.

**Material examined. Project “BIOTA - Araçá”:** São Sebastião Channel - Araçá Bay (state of São Paulo). 23°49'11,4"S – 45°24'13,0"W; sublittoral; station 68(1)Mc; 19 Feb 2013; 1 spec. 23°48'56,0"S – 45°23'41,5"W; shallow subtidal; station 148vV; 14 Oct 2013; 0.5 m; 2 specs. 23°48'52,1"S – 45°24'23,5"W; tidal flat; station 124(2); 17 Sep 2013; 1 spec. 23°49'20,1"S – 45°24'10,3"W; shallow subtidal; station 145vV; 14 Oct 2013; 0.5 m; 3 specs. 23°48'58,1"S – 45°24'22,0"W; tidal flat; station 127(4); 17 Sep 2013; 1 spec. 23°48'52,1"S – 45°24'23,5"W; tidal flat; station 124(3); 17 Sep 2013; 1 spec. **Project “HABITATS”:** Campos Basin (states of Rio de Janeiro and Espírito Santo). Paraíba do Sul river



**mouth:** 21°40'23"S – 40°58'23"W; sublittoral; 18 m; station HAB 17/FOZ 05/R1; 19 Jul 2009; 1 spec. 22°01'10"S – 40°31'53"W; sublittoral; 49 m; station HAB 13/FOZ 23/R3; 13 Mar 2009; 4 specs. 22°01'10"S – 40°31'53"W; sublittoral; 49 m; station HAB 13/FOZ 23/R2; 12 Mar 2009; 2 specs. 22°01'09"S – 40°31'55"W; sublittoral; 49 m; station HAB 17/FOZ 23/R2; 24 Jul 2009; 1 spec. **Continental Shelf:** 22°1'46"S – 40°44'56"W; sublittoral; 27 m; station HAB 11/E 01/R1; 26 Feb 2009; 2 specs. 22°1'45"S – 40°44'52"W; sublittoral; 28 m; station HAB 11/E 01/R3; 26 Feb 2009; 3 specs. 22°1'46"S – 40°44'55"W; sublittoral; 28 m; station HAB 17/E 01/R3; 18 Jul 2009; 1 spec. 22°6'56"S – 40°38'58"W; sublittoral; 53 m; station HAB 11/E 02/R1; 26 Feb 2009; 1 spec. 22°8'9"S – 40°27'27"W; sublittoral; 65 m; station HAB 11/E 03/R1; 23 Feb 2009; 2 specs. 22°8'9"S – 40°27'27"W; sublittoral; 66 m; station HAB 16/E 03/R2; 04 Jul 2009; 1 spec. 21°42'53"S – 40°10'16"W; sublittoral; 98 m; station HAB 13/H 04/R2; 09 Mar 2009; 1 spec. 22°1'45"S – 40°44'51"W; sublittoral; 28 m; station HAB 11/E 01/R2; 26 Feb 2009; 6 specs. 22°37'31"S – 41°21'51"W; sublittoral; 53 m; station HAB 11/B 02/R3; 27 Feb 2009; 2 specs. 22°6'44"S – 40°54'44"W; sublittoral; 30 m; station HAB 17/D 01/R3; 17 Jul 2009; 9 specs. 22°1'46"S – 40°44'51"W; sublittoral; 28 m; station HAB 17/E 01/R1; 18 Jul 2009; 16 specs. 22°1'47"S – 40°44'52"W; sublittoral; 28 m; station HAB 17/E 01/R2; 18 Jul 2009; 16 specs.

**Description.** All examined individuals were incomplete ranging from 0.2 to 6.8 mm wide on thorax, and from 0.1 to 5.0 mm wide on abdomen. The longer specimen bears 160 chaetigers. Body robust; thorax wider than abdomen. Red in life and light brown in ethanol. Prostomium conical, with a rounded tip. Proboscis globular-shaped, with soft and robust papillae (Fig. 3C). Eyespots absent. Peristomium achaetous, longer than first two thoracic chaetigers. Peristomium and all thoracic chaetigers with strongly tessellated epithelium (Figs. 1A, B; 2A, B, C and 3A). Smooth nuchal organs (Figs. 1B; 2B, D and 3B). First chaetiger biramous. Dorsal bundles of chaetae with the same distance of each other along the thorax. All chaetigers biannulate with shallow intra segmental grooves (Figs. 1A, B; 2A, B, C and 3A). Thorax flattened dorso-ventrally, with the dorsal region smaller than the ventral one (Figs. 1A; 2B and 3A). Lateral organs and genital pores not observed on thorax. Transition between thorax and abdomen well distinct. Abdominal chaetigers with hooded hooks in neuropodia only, notopodial hooks absent. Dorso-lateral globular lateral organs present along the entire abdomen (Figs. 1A, B, C; 2E and 3D). Nephridia present along the entire abdomen, posterior to the lateral organ, observed by transparency of the body wall. Reduced notopodial lobes

present on first 3 – 6 abdominal chaetigers, with decreasing size (Figs. 1C and 2G). Abdomen with ventrally neuropodial expansions beginning between chaetigers 17 and 23 (Figs. 1D; 2E and 3E). Neuropodial fascicles with 10 to 97 hooded hooks, ventro-lateral, not reaching the middle of the chaetiger (Figs. 1E and 2F). Anterior hooded hooks (first nine abdominal chaetigers) with one row of 4 – 5 teeth above main fang, main fang straight, long anterior shaft, slightly developed shoulder, narrow and smooth hood (Fig. 2H). Posterior hooded hooks, after 20<sup>th</sup> chaetiger, similar to the anterior ones, except by the hood, which becomes larger and with a fringed margin (Figs. 2I and 3F, G). Branchiae absent. Pygidium unknown.

**Methyl green staining pattern.** Thorax staining uniformly, except for chaetiger 11, which presents a darker band on the third part of the segment (Fig. 2C). Abdominal segments with a dark stain on the notopodia due to a glandular area (Figs. 1B, C, D and 2C, E).

**Variation.** Specimens of the Araçá Bay ranged from 0.9 to 5.0 mm wide on thorax, notopodial lobes between the 2<sup>nd</sup> and 4<sup>th</sup> abdominal chaetigers, and appearance of neuropodial expansions between chaetigers 17 and 21. Specimens of the Paraíba do Sul river mouth ranged from 1.5 to 3.2 mm wide on thorax, notopodial lobes between 2<sup>nd</sup> and 5<sup>th</sup> abdominal chaetigers, and appearance of neuropodial expansions after chaetiger 19. Specimens of the Continental Shelf ranged from 0.2 to 5.0 mm wide on thorax, notopodial lobes between 2<sup>nd</sup> and 6<sup>th</sup> abdominal chaetigers, and appearance of neuropodial expansions between chaetigers 17 and 23. Individuals below 0.8 mm wide without lobes.

**Remarks.** The specimens observed here were consistent with those described by Hartman (1947), except by reduced notopodial lobes on first 3 – 6 abdominal chaetigers, anterior hooks with a narrow and smooth hood and posterior ones with larger hoods with a fringed margin, and neuropodial expansions beginning between chaetigers 17 and 23. According to Hartman's description, the neuropodial expansions begin between thirtieth and fortieth chaetigers, however, a variation of this character was verified, and maybe it is size dependent. Regarding the description of the hooded hooks, the ornamentation of the hoods is better visible under scanning electron microscope, maybe the author did not have access to this procedure and was not able to detect the difference.

**Habitat.** From intertidal to sublittoral regions (0 – 149 m).

**Type locality.** Gulf of California, Pacific Ocean.

**Distribution.** Western Atlantic: North Carolina to the Gulf of Mexico; South Atlantic Ocean: Brazil (states of Espírito Santo, Rio de Janeiro and São Paulo).

***Rashgua* sp. n. A**

(Figures 4 – 6; Table 1)

**Material examined. Project “HABITATS”: Campos Basin (states of Rio de Janeiro and Espírito Santo).** **Slope:** 21°11'4"S – 40°9'10"W; sublittoral; 984.3 m; station HAB 3/I 08/R1; 14 May 2008; 1 spec. 22°36'27"S – 40°22'29"W; sublittoral; 697.9 m; station HAB 6/D 07/R1; 25 Jun 2008; 1 spec. 22°36'25"S – 40°22'28"W; sublittoral; 698.1 m; station HAB 8/D 07/R1; 29 Jan 2009; 1 spec.

**Description.** Examined individuals were incomplete measuring from 1.8 to 2.4 mm wide on thorax and from 1.0 to 2.4 mm wide on abdomen. Body robust, rounded. Thorax wider than abdomen. Light brown in ethanol. Prostomium triangular, with a pointed tip. Proboscis not everted. Eyespots absent. Peristomium achaetous, longer than first thoracic chaetiger (Figs. 4A; 5A and 6A, B). Nuchal organs not everted. Peristomium and all thoracic chaetigers with tessellated epithelium. Anterior segments with a net aspect and posterior ones with vertical lines, forming a rectangular pattern (Figs. 4A; 5A and 6A). First chaetiger biramous. Right and left dorsal bundles of chaetae with the same distance of each other along the thorax. Chaetigers biannulate with shallow intra segmental grooves between chaetigers 1 – 4 and deep intra segmental grooves between chaetigers 5 – 11 (Figs. 4A; 5A and 6A). Lateral organs absent on thorax. Genital pores observed on the intersegmental division between chaetigers 6 – 11 (Fig. 6C). Transition between thorax and abdomen subtle. Abdominal chaetigers with hooded hooks in neuropodia only. Dorso-lateral globular lateral organs present along the entire abdomen (Figs. 4A, B and 5C). Presence of two triangular notopodial lobes on each chaetiger (Figs. 4A, B and 5C). Notopodial hooks absent. Abdomen with neuropodial lobes slightly expanded ventrally and globulous dorso-laterally (Fig. 5D). Neuropodial fascicles with 20 to 50 hooded hooks, very close to each other on the middle of the chaetiger (Figs. 4C and 5D). Hooded hooks with one row of 4 teeth above main fang, main fang very robust, moderate anterior shaft, slightly developed shoulder, and short hood. (Fig. 5B). Branchiae absent. Pygidium unknown.

**Methyl green staining pattern.** Abdominal segments with an “X” stain pattern in notopodia due to a glandular area (Figs. 4A, B and 5C).

**Remarks.** *Rashgua* sp. n. A differs from *R. rubrocincta* by having neuropodial expanded lobes and it also differs from *R. branchiatus* and *R. lobatus* because its lobes are just slightly expanded, globulous dorso-laterally, in contrast with long and thin digitates lobes of the latter species.

**Habitat.** From deep sublittoral regions (697.9 – 984.3 m).

**Distribution.** South Atlantic Ocean: Brazil (states of Espírito Santo and Rio de Janeiro).

### ***Rashgua* sp. B**

(Figures 7 – 9; Table 1)

**Material examined.** Project “AMBES”: Espírito Santo Basin (state of Espírito Santo).

**Continental Shelf:** 21°3'27"S – 40°22'59"W; sublittoral; 36 m; station AMB 7/A 02/R1; 22 Jan 2012; 1 spec.

**Description.** The examined individual was incomplete measuring 2.9 mm wide on thorax and 2.1 mm wide on abdomen. Body robust, flattened dorso-ventrally. Thorax wider than abdomen. Light brown in ethanol. Prostomium squared, robust. Proboscis globular-shaped, with soft and robust papillae (Figs. 7A; 8A, D and 9A). Eyespots present in a semilunar shape (Figs. 7A and 8A). Peristomium achaetous, longer than all thoracic chaetigers (Figs. 7A; 8A and 9A). Nuchal organs not everted. First chaetiger biramous. All thoracic chaetigers with strongly tessellated epithelium (Figs. 7A; 8A, D and 9A). Right and left dorsal bundles of chaetae with the same distance of each other along the thorax. All chaetigers biannulate with deep intra segmental grooves (Figs. 7A; 8A and 9A). Lateral organs absent on thorax. No genital pores observed. Transition between thorax and abdomen well distinct. Abdominal chaetigers with hooded hooks in neuropodia only. Dorso-lateral globular lateral organs conspicuous only on the first abdominal chaetiger (Fig. 8A). Notopodial lobes absent on abdomen (Figs. 7A; 8A and 9A). Notopodial hooks absent. Abdomen with slightly expanded and continuous neuropodial lobes, with just a small depression on the middle of the abdomen (Figs. 7B; 8B, D and 9B). Neuropodial fascicles with approximately 50 hooded hooks, very close to each other on the middle of the chaetiger (Figs. 7B; 8B, D and 9B). Hooded hooks

with one row of 8 – 9 teeth above main fang, main fang robust, moderate anterior shaft, slightly developed shoulder, and very short hood. (Figs. 8C and 9C). Branchiae absent. Pygidium unknown.

**Methyl green staining pattern.** Abdominal segments with a dark band on the notopodia due to a glandular area (Fig. 8A).

**Remarks.** *Rashgua* sp. B resembles *R. rubrocincta* by the absence of expansions on the neuropodial lobes, however, it differs from the latter by the absence of notopodial lobes and by having 8 – 9 teeth above main fang, in contrast with *R. rubrocincta*, which has notopodial lobes and only one tooth above main fang. Additionally, *Rashgua* sp. B has eyespots, in contrast with all *Rashgua* species described.

**Habitat.** From sublittoral regions (36 m).

**Distribution.** South Atlantic Ocean: Brazil (state of Espírito Santo).

## Acknowledgments

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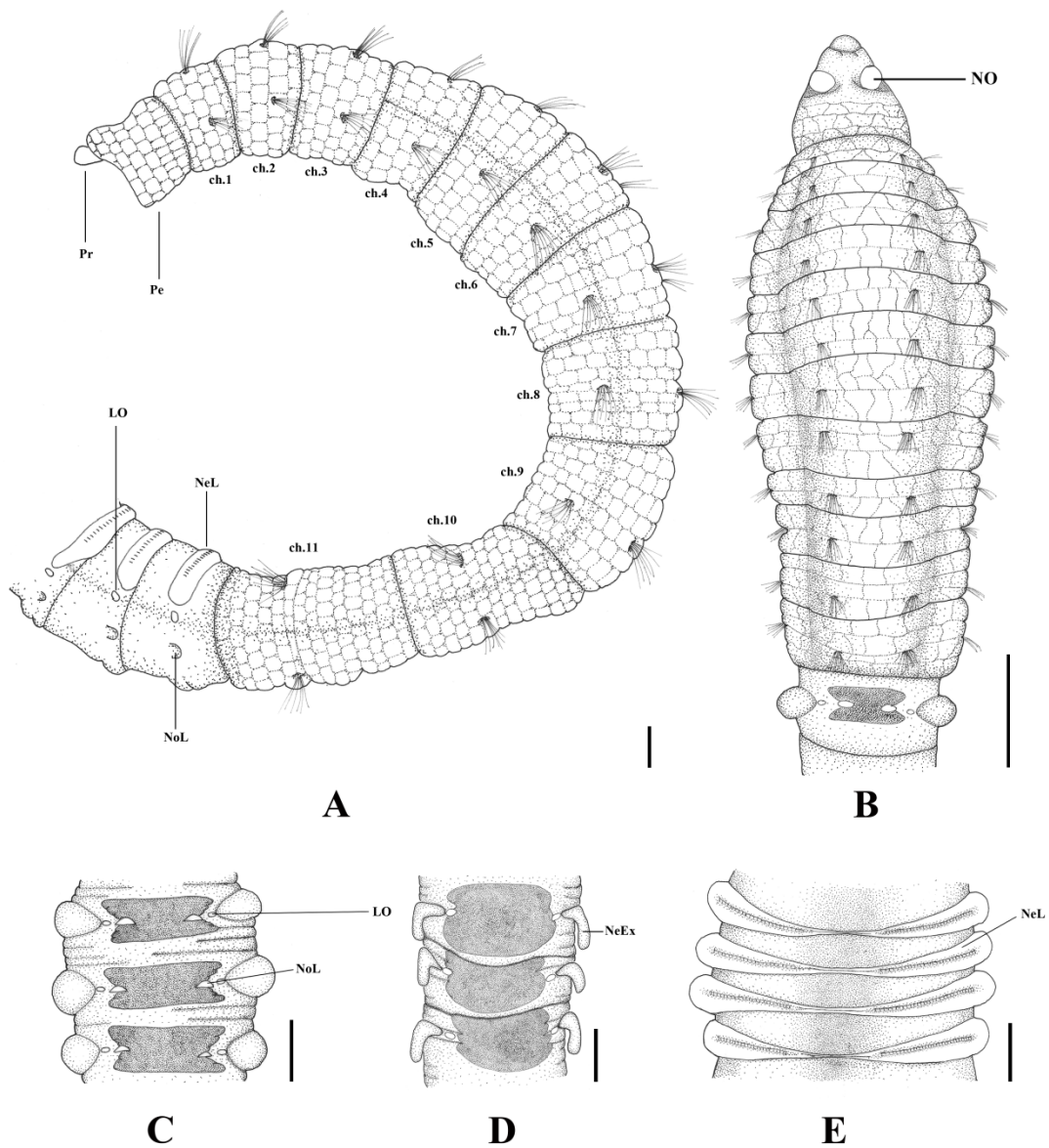
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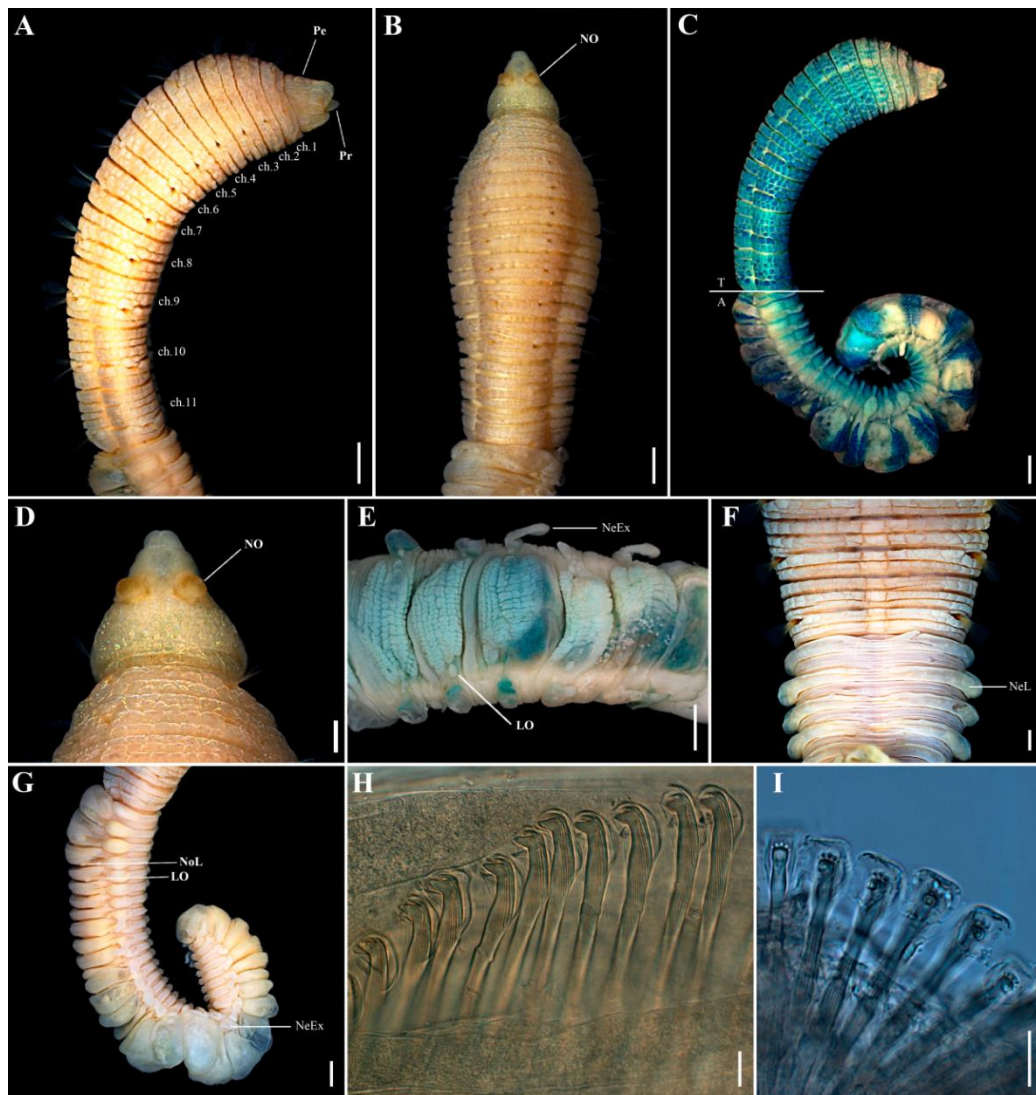


**Table 1.** Comparison among *Rashgua* species.

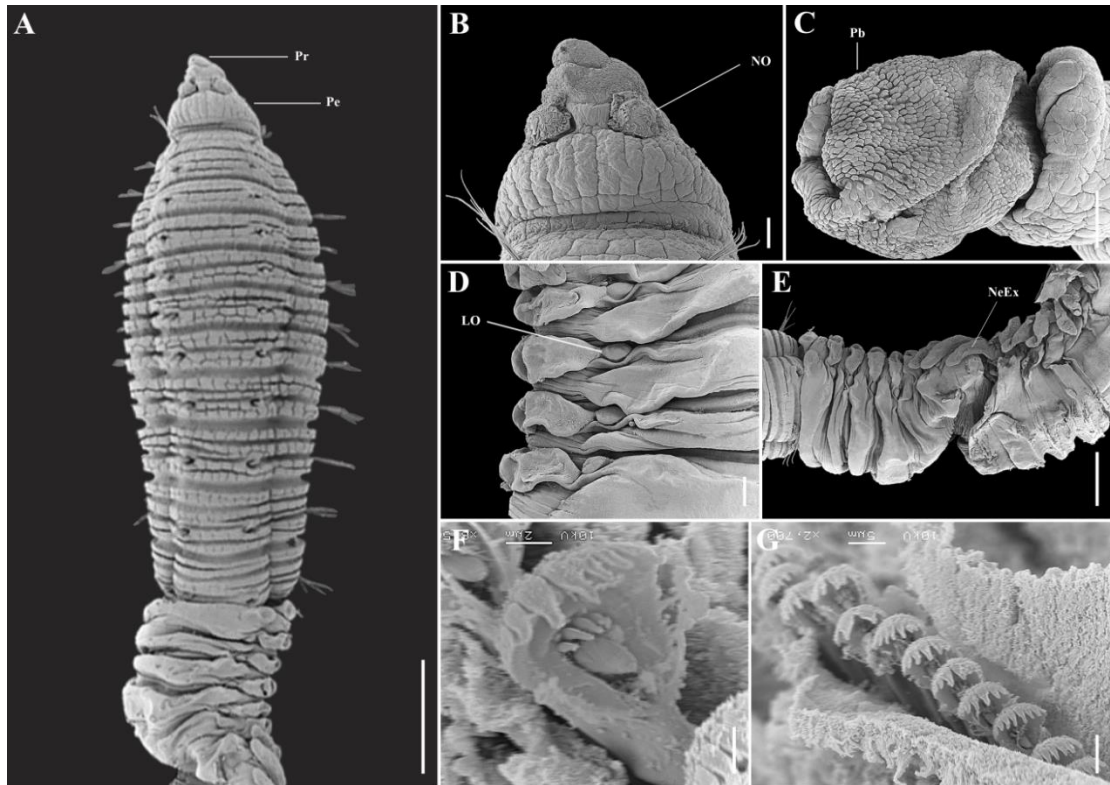
| Species  | Prostomium | Eyes    | Notopodial hooks                        | Notopodial lobes                                     | Neuropodial expansions  | Abdominal hooks           | Size range (width, mm) | Depth (m)     | Type locality                                       | References   |
|--|------------|---------|---|--|---|---------------------------|------------------------|---------------|---|--|
| <i>Rashgua branchiatus</i><br>Green, 2002          | Conical    | absent  | present on first 2 abdominal chaetigers | present reduced; throughout the abdomen              | present digitated; on anterior abdomen (between chaetigers 15 - 33) | 5 teeth in 2 rows         | 1.4 - 3.0              | 31 - 45       | Andaman Sea, Thailand                               | Green, 2002 (Fig. 21A,H)                                       |
| <i>Rashgua lobatus</i><br>(Hartman, 1947)          | Conical    | absent  | absent                                  | present reduced; on first 3 - 6 abdominal chaetigers | present digitated; on anterior abdomen (between chaetigers 13 - 23) | 4-5 teeth in a single row | 0.2 - 6.8              | 0 - 149       | Gulf of California, Pacific Ocean                   | Hartman, 1947 (Plate 51, Figs. 1-5); current study (Figs. 1-3) |
| <i>Rashgua rubrocincta</i><br>Wesenberg-Lund, 1949 | Conical    | absent  | absent                                  | present reduced; throughout the abdomen              | present globulous; throughout the abdomen                           | -                         | 6.0                    | 7 - 22        | Persian Gulf  | Wesenberg-Lund, 1949 (Figs. 37-38)                             |
| <i>Rashgua</i> A sp.n.                             | Triangular | absent  | absent                                  | present triangular; throughout the abdomen           | present globulous; throughout the abdomen                           | 4 teeth in a single row   | 1.8 - 2.4              | 697.9 - 984.3 | Campos Basin, Brazil, South Atlantic Ocean          | Current study (Figs. 4-6)                                      |
| <i>Rashgua</i> sp.B                                | Squared    | present | absent                                  | absent   | absent  | 8-9 teeth in a single row | 2.9                    | 36            | Esprito Santo Basin, Brazil, (South Atlantic Ocean) | Current study (Figs. 7-9)                                      |



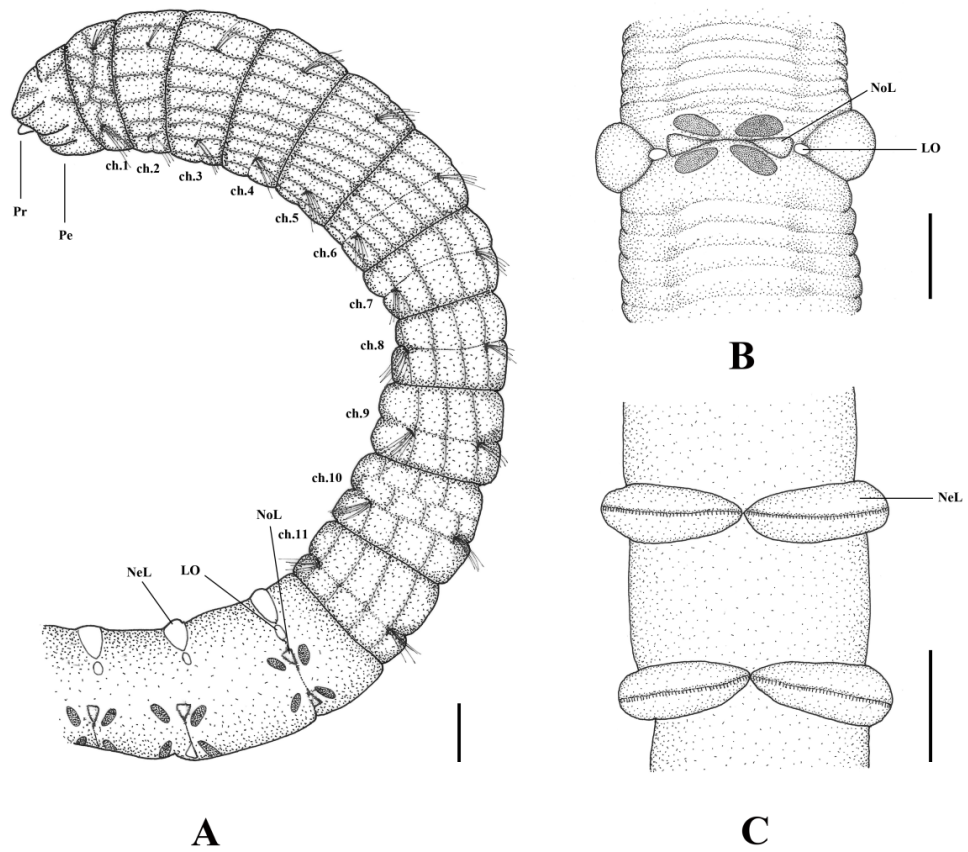
**Fig. 1.** *Rashgua lobatus* (A) Thoracic region, lateral view; (B) Thoracic region, dorsal view; (C) Anterior abdominal region, dorsal view; (D) Posterior abdominal region, dorsal view; (E) Anterior abdominal region, ventral view. Pr: prostomium. Pe: peristomium. NO: nuchal organ. LO: lateral organ. NoL: notopodial lobe. NeL: neuropodial lobe. NeEx: neuropodial expansion. Ch: chaetiger. Scale bars: A, B, C, D, E, 1 mm.



**Fig. 2.** *Rashgua lobatus* (A) Thoracic region, lateral view; (B) Thoracic region, dorsal view; (C) Methyl green staining pattern (MGSP); (D) Anterior end with nuchal organ everted; (E) Abdominal region, dorsal view, (MGSP); (F) Abdominal region, ventral view; (G) Abdominal region, lateral view; (H) Anterior abdominal hooded hooks, lateral view; (I) Posterior abdominal hooded hooks, frontal view. Pr: prostomium. Pe: peristomium. NO: nuchal organ. T: thorax. A: abdomen. LO: lateral organ. NeEx: neuropodial expansion. NoL: notopodial lobe. NeL: neuropodial lobe. Ch: chaetiger. Scale bars: A, E, C, 1.0 mm; B, F, G, 0.5 mm; H, I, 10  $\mu$ m.



**Fig. 3.** *Rashgua lobatus*, SEM. (A) Thoracic region, dorsal view; (B) Nuchal organ; (C) Anterior end, lateral view; (D) Anterior abdominal region, dorsal view; (E) Abdominal region, dorso-lateral view; (F) Abdominal hooded hook, frontal view; (G) Abdominal hooded hooks. Pr: prostomium. Pe: peristomium. NO: nuchal organ. Pb: proboscis. LO: lateral organ. NeEx: neuropodial expansion. Scale bars: A, 1 mm; B, D, 0.1 mm; C, E, 0.5 mm; F, 2  $\mu$ m; G, 5  $\mu$ m.



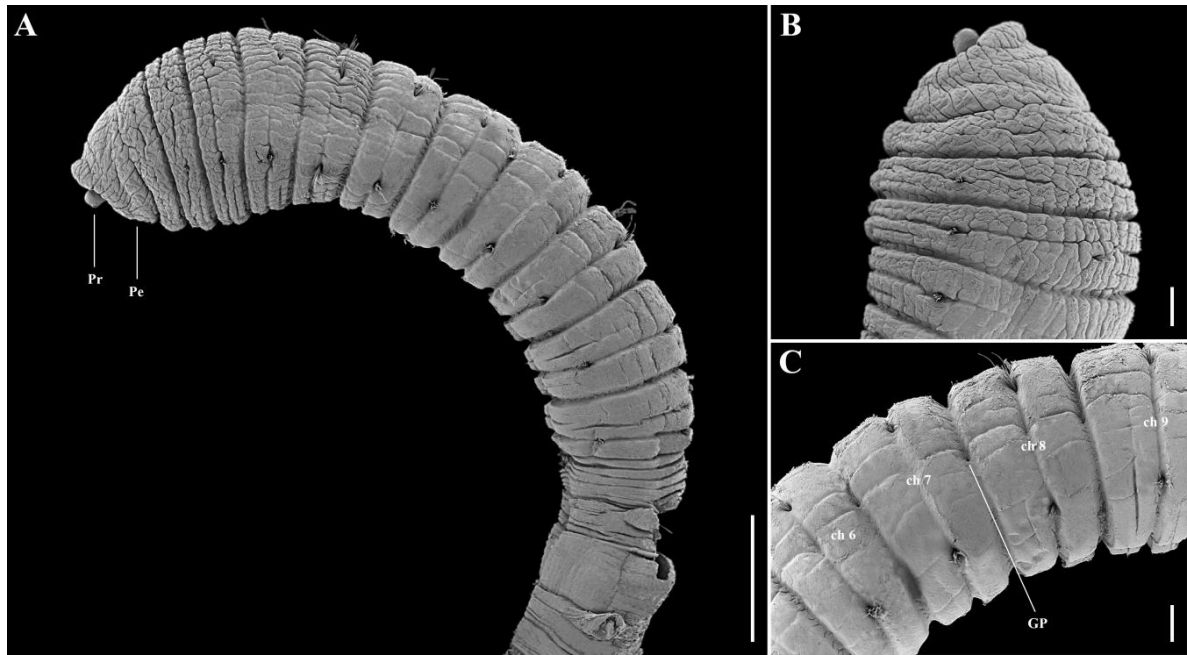
**Fig. 4.** *Rashgua* sp. A (A) Thoracic region, lateral view; (B) Notopodial abdomen, dorsal view; (C) Neuropodial abdomen, ventral view. Pr: prostomium. Pe: peristomium. LO: lateral organ. NoL: notopodial lobe. NeL: neuropodial lobe. Ch: chaetiger. Scale bars: A, B, C, 1 mm.



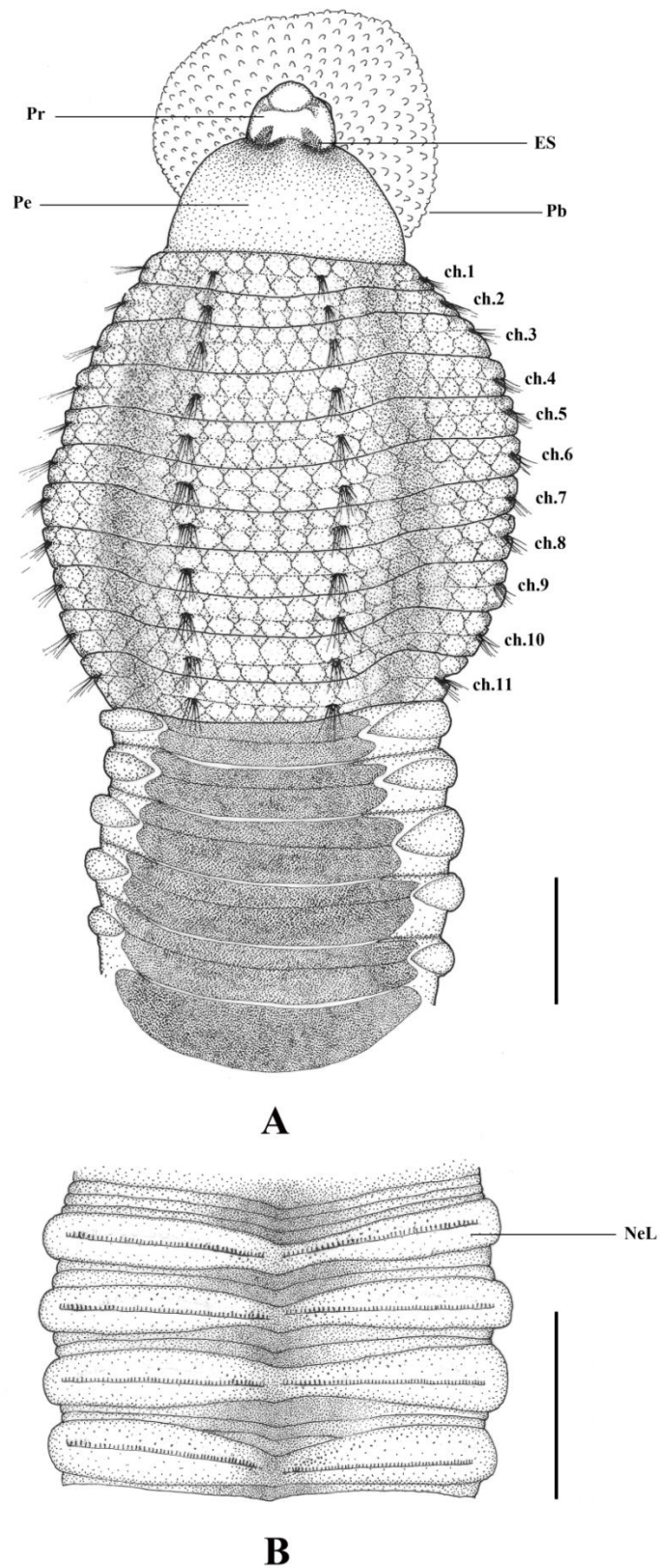


**Fig. 5.** *Rashgua* sp. A (A) Thoracic region, lateral view; (B) Abdominal hooded hooks; (C) Abdominal region, dorsal view; (D) Abdominal region, ventral view. Pr: prostomium. Pe: peristomium. LO: lateral organ. NoL: notopodial lobe. NeL: neuropodial lobe. Ch: chaetiger. Scale bars: A, 1 mm; B, 10  $\mu$ m; C, D, 0.2 mm.

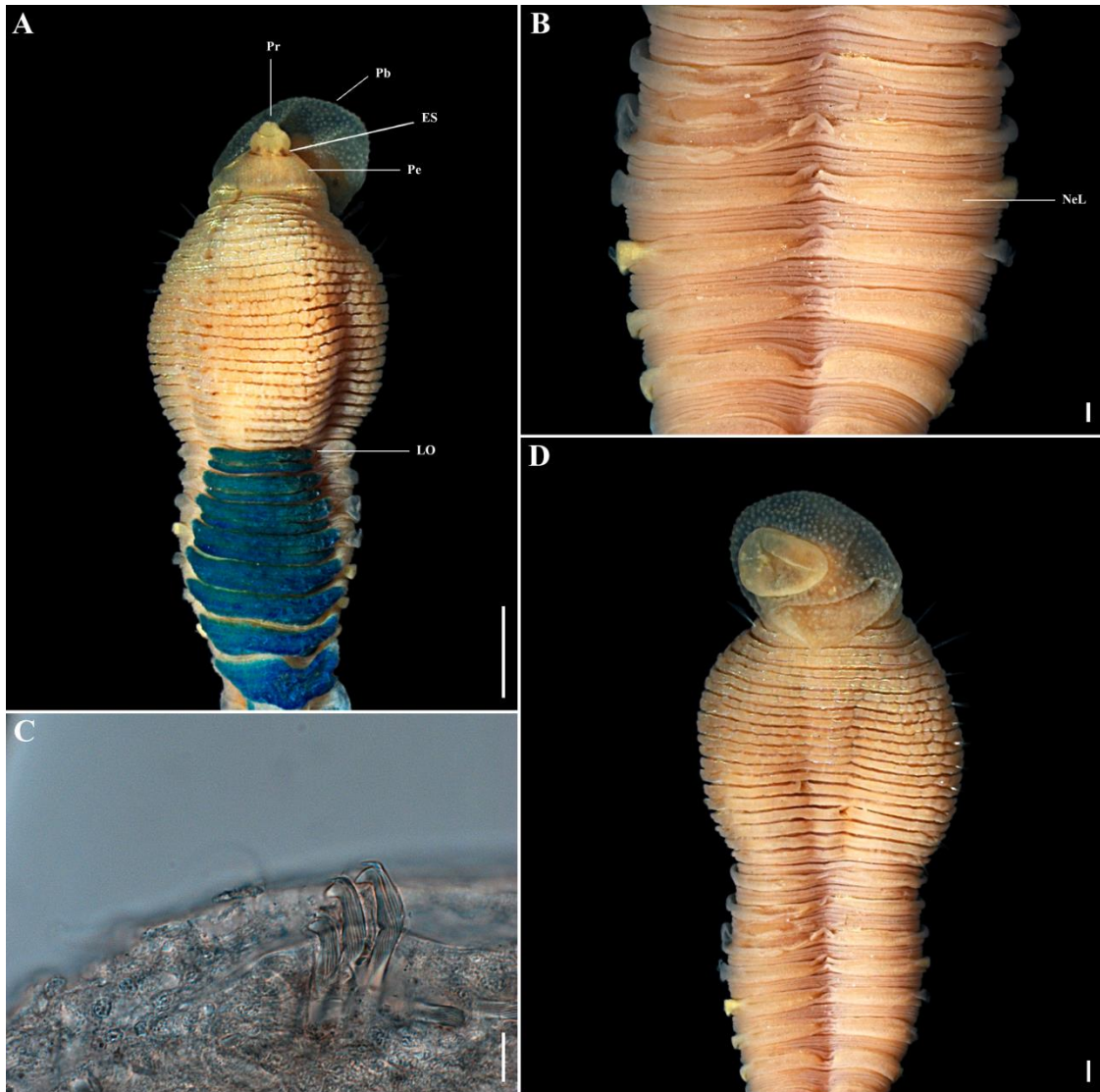




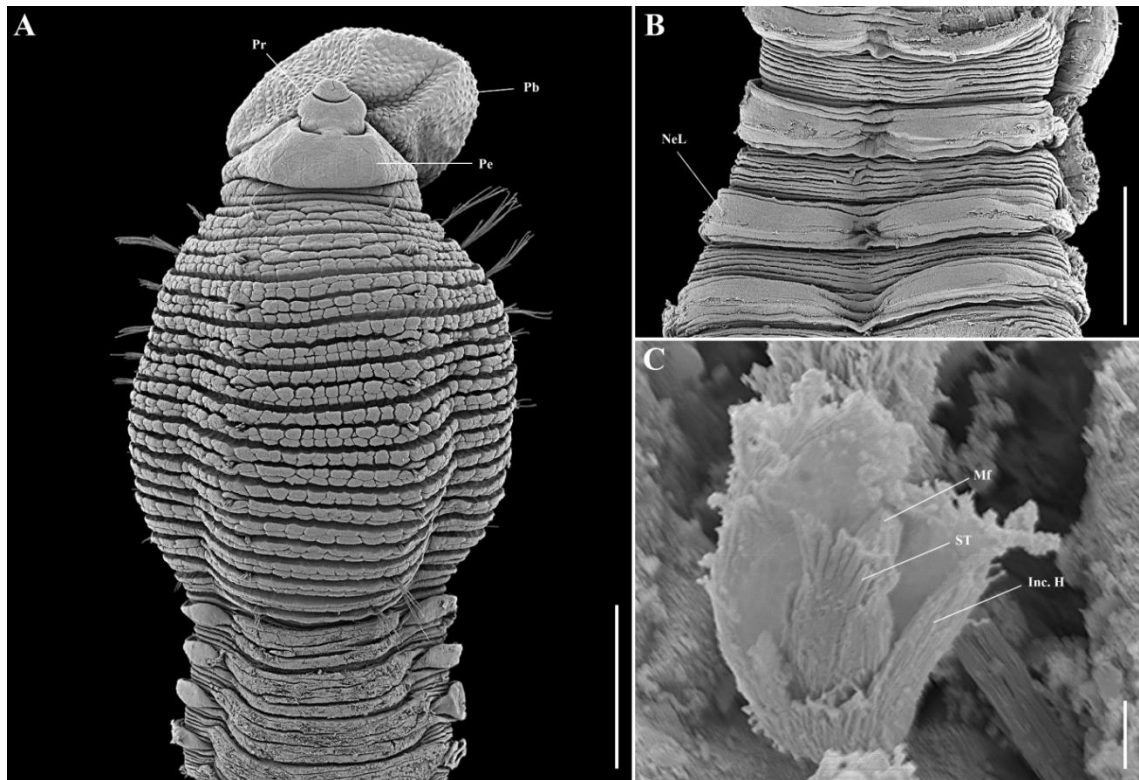
**Fig. 6.** *Rashgua* sp. A, SEM. (A) Thoracic region, lateral view; (B) Anterior end, lateral view; (C) Medium thoracic region, lateral view. Pr: prostomium. Pe: peristomium. GP: genital pore. Ch: chaetiger. Scale bars: A, 1 mm; B, C, 0.2 mm.



**Fig. 7.** *Rashgua* sp. B (A) Thoracic region, dorsal view; (B) Abdominal region, ventral view. Pr: prostomium. Pe: peristomium. Pb: proboscis. NeL: neuropodial lobe. Ch: chaetiger. ES: eyespots. Scale bars: A, B, 1 mm.



**Fig. 8.** *Rashgua* sp. B (A) Thoracic and abdominal regions, dorsal view, abdomen with methyl green; (B) Abdomen, ventral view; (C) Hooded hooks, lateral view; (D) Thoracic and abdominal regions, ventral view. Pr: prostomium. Pe: peristomium. Pb: proboscis. LO: lateral organ. NeL: neuropodial lobe. ES: eyespots. Scale bars: A, 1 mm; B, 0.1 mm; C, 10 µm; D, 0.2 mm.



**Fig. 9.** *Rashgua* sp. B, SEM. (A) Thoracic and abdominal regions, dorsal view; (B) Abdomen, ventral view; (C) Hooded hook, top view. Pr: prostomium. Pe: peristomium. Pb: proboscis. NeL: neuropodial lobe. Mf: main fang. ST: superior teeth. Inc. H: incomplete hood. Scale bars: A, 1 mm; B, 0.5 mm; C, 2  $\mu$ m.

## **CAPÍTULO 5**

**Species of *Scyphoproctus* Gravier, 1904 (Annelida: Capitellidae)**

**along the Brazilian coast with description of three new species**

## **Species of *Scyphoproctus* Gravier, 1904 (Annelida: Capitellidae) along the Brazilian coast with description of three new species**

**Abstract.** Capitellids of the genus *Scyphoproctus* are most readily identified by the distinctive pygidial region, generally consisting of a number of segments fused with the pygidium, and bearing acicular spines. To date, there are 17 valid species, however, only one species have been recorded from Brazil. The aim of this study was to investigate the diversity of the genus occurring along the Brazilian coast. Three new species of *Scyphoproctus* have been found in samples collected on a variety of habitats at the Northeast and Southeast Brazil. The new species are herein described and compared to other species of the genus. The current state of knowledge on the genus and a taxonomic key are given.

**Key words:** Polychaeta, morphology, intertidal region, rocky shore, Continental Shelf, Brazil

### **Introduction**

Capitellids of the genus *Scyphoproctus* Gravier, 1904 are most readily identified by the distinctive pygidial region. Generally it comprises a dorsally flattened anal plaque consisting of a number of segments fused with the pygidium, and bearing acicular spines, however, some species present a poorly developed anal plaque, which can or cannot bears acicular spines.

The original diagnosis of the genus was made by Gravier (1904). Day (1965) emended the diagnosis to include the variation of the number of thoracic chaetigers and pointed out the appearance of hooded hooks on thorax. However, the range of thoracic segments had been firstly reported by Rangarajan (1963) with the description of *Scyphoproctus variabilis*. Doyle (1991) included the occurrence of lateral organs and discussed the range of segments in the pre-pygidial region, the number of segments fused with the pygidium to form the plaque and the dispersion of acicular spines on the plaque. Green (2002) pointed out that other species also vary in the number of thoracic chaetigers with capillaries and determined several new characters of the anal plaque to identify *Scyphoproctus* species, such as number of acicular spines per segment, position and size of anus, shape of anal plaque, length and fusion of anal cirri, in addition to the methyl green staining pattern of the specimens.



Recently, with the description of *S. edmondsoni*, Magalhães & Bailey-Brock (2012) synonymized the genus *Pulliella* with *Scyphoproctus*, because this species also has an incompletely developed anal plaque. They discussed that the development of an anal plaque, complete or incomplete, is an exclusive feature and numbers of acicular spines should be considered species-level characters. The authors also emended the diagnosis to include species with 11 thoracic segments (nine chaetigers) and to differentiate among species with pygidium expanded as a poorly or well-developed anal plaque.

Currently, there are 17 valid species, *Scyphoproctus djiboutiensis* Gravier, 1904, *S. armatus* (Fauvel, 1929), *S. oculatus* Reish, 1959, *S. platyproctus* Jones, 1961, *S. variabilis* Rangarajan, 1963, *S. pseudoarmatus* (Silva, 1965), *S. pullielloides* Hartmann-Schröder, 1965, *S. steinitzii* Day, 1965, *S. somalus* Cantone, 1976, *S. ornatus* Hartmann-Schröder, 1979, *S. aciculatus* Mohammad, 1980, *S. guadalupensis* Gillet, 1986, *S. towraiensis* Doyle, 1991, *S. fasciculatus* Green, 2002, *S. glabrus* Green, 2002, *S. lumenalis* Green, 2002, and *S. edmondsoni* Magalhães & Bailey-Brock, 2012.

Treadwell (1901) described *Dasybranchus rectus* and later Treadwell (1939) synonymized it to *Scyphoproctus rectus*, however, Hartman (1947) questioned its generic status because the specimen was incomplete and there was no mention to the presence of an anal plaque. *Scyphoproctus gravieri* Okuda, 1940, sampled at Kakinada, Okinawa, was described from a single posterior fragment according to the characteristics of the anal plaque, thus, this species must be classified as *species inquirenda* until new collections and formal description of the entire specimen be made. *Scyphoproctus somalus* was first re-described by Paxton et al. (1984) and posteriorly by Doyle (1991).

Based on the drawings and description of *Scyphoproctus oculatus*, this species lacks the second achaetous segment posterior to peristomium; however, it bears a well-developed anal plaque, confirming that it belongs to the genus. *Scyphoproctus* species may present a complete or incomplete inter-segmental groove traversing the achaetous segment (not the peristomium), which may or may not be conspicuous, and maybe misinterpreted by the author. A further examination of the type material would be helpful to solve this issue.

The capitellid fauna from Brazil is still poorly known, up to now only one species of this genus has been recorded, *Scyphoproctus djiboutiensis*, this way the aim of this study was to investigate the diversity of the genus occurring along the Brazilian coast. Here, we describe three new species and a taxonomic key to the valid species is given.

## Material and Methods

### *Material examined*

The specimens were collected at different habitats along the Brazilian coast, under the scope of eight independent projects:

- 1) FDP “Beach Fauna” (1995 – 1997) ( $23^{\circ}43' - 23^{\circ}49'S / 45^{\circ}27' - 45^{\circ}24'W$ ), aimed to study the macrofauna of 13 sandy beaches along the São Sebastião Channel, state of São Paulo, Southeast Brazil;
- 2) REVIZEE/South Score/Benthos/MMA “Program of the Evaluation of the Living Resources of the Exclusive Economic Zone” (1997 – 1998) ( $21^{\circ} - 34^{\circ}S / 40^{\circ} - 52^{\circ}W$ ), focusing on the biodiversity of the Continental Shelf and the Slope (60 – 800 m) of the south-eastern and southern parts of the Brazilian Exclusive Economic Zone (ZEE), between the states of Rio de Janeiro and Rio Grande do Sul, Southeast and South Brazil (for details see Amaral *et al.*, 2004);
- 3) BIOPORE “Population and Reproductive Biology of Invertebrates” (2006 – 2007) ( $23^{\circ}37'S - 45^{\circ}23'W$ ), aimed to study the biology of invertebrates inhabiting two soft bottom mussel beds of Camaroeiro and Cidade beaches, Caraguatatuba Bay, state of São Paulo, Southeast Brazil (for details see Silva, 2013);
- 4) HABITATS/CENPES/PETROBRAS – “Campos Basin Environmental Heterogeneity” (2008 – 2009) ( $21^{\circ} - 24^{\circ}S / 38^{\circ} - 45^{\circ}W$ ), with focus on soft bottoms of four habitats (12 – 3301 m), the Paraíba do Sul river mouth, the Continental Shelf, the Slope, and the canyons Almirante Câmara (CANAC) and Grussaí (CANG), of the Campos Basin, states of Espírito Santo and Rio de Janeiro, Southeast Brazil (for details see Lavrado and Brasil, 2010);
- 5) BIOPOL-NE “Diversity of Polychaeta (Annelida) on Hard Substrates off the Northeastern Brazil” (2009 – 2010) ( $6^{\circ} - 8^{\circ}S / 34^{\circ} - 35^{\circ}W$ ), aimed to collect on rocky shores, tufts of algae, mussel beds, colonies of sponges and ascidians, small pieces of sabellariid reefs, and similar substrates, along beaches off the states of Paraíba and Pernambuco, Northeast Brazil (for details see Paresque, 2014);
- 6) AMBES – “Environmental Characterization of the Espírito Santo Basin” (2010 – 2013) ( $18^{\circ} - 21^{\circ}S / 37^{\circ} - 40^{\circ}W$ ), with focus on soft bottoms of four habitats (25 – 3000 m), the Rio Doce river mouth, the Continental Shelf, the Slope, and the canyons

Watu Norte (CANWN) and Rio Doce (CAND) of the Espírito Santo Basin, state of Espírito Santo, Southeast Brazil;

- 7) OIL SPILL (2013 – 2014) (23°48'S – 45°4'W), aimed the monitoring of the macrofauna of sandy beaches affected by an oil spill accident at São Sebastião Channel, northern coast of the state of São Paulo, Southeast Brazil;
- 8) BIOTA/FAPESP - Araçá “Biodiversity and functioning of a subtropical coastal ecosystem: subsidies for integrated management” (2012 – 2016) (23°48'S – 45°4'W), the aim of this project was to study the fauna of different habitats (0 – 25 m): mangrove, tidal and subtidal region of the Araçá Bay, São Sebastião Channel, northern coast of the state of São Paulo, Southeast Brazil (for details see Amaral *et al.*, 2016).

### ***Morphological data***

Specimens were examined using optical microscopy (Zeiss Axio Imager M2), stereomicroscopy (Zeiss Axio Zoom v16) and, in some cases, with a Scanning Electron Microscope (SEM). Line drawings were made with a camera lucida attached to a compound microscope and afterwards hand drawn with Indian ink. Measurements were taken with an ocular micrometer that was calibrated with a stage micrometer. The body length was measured from the anterior margin of the prostomium to the tip of the pygidium, while the width was measured at the widest segment, excluding the chaetae. For SEM images, specimens were dehydrated in a series of ethanol solutions with progressively increasing concentrations (75 – 100%), critical-point-dried with a Balzers CPD 30 (temperature 37 °C and pressure 70 kg/cm<sup>2</sup>), mounted on stubs, covered with a layer of 10 – 20 nm of gold, and observed under the SEM at the Laboratório de Microscopia Eletrônica, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP) using the JEOL JSM-5800 LV Scanning Electron Microscope.

The nomenclature used for morphology of the hooded hooks and the characters of the anal plaque follow the suggested by Green (2002). The characters used for hooks are the shape of main fang, number of teeth and rows, and the size, format, and texture of the hood; and the characters used for the anal plaque are shape of the plaque, position and number of acicular spines, number of pre-pygidial segments, size of cirri and anus, and presence or absence of a median membrane between cirri.

The material was deposited at the Museu de Zoologia, Universidade Estadual de Campinas (ZUEC).

### **Taxonomic Account**

#### **Family Capitellidae Grube, 1862**

#### **Genus *Scyphoproctus* Gravier, 1904**

**Synonyms** *Pulliella* Fauvel, 1929. Synonymized by Magalhães & Bailey-Brock (2012).

Type species: *Scyphoproctus djiboutiensis* Gravier, 1904.

Type locality: Red Sea.

**Diagnosis** (emended by Magalhães & Bailey-Brock, 2012). Thorax with 11 – 16 segments, including two achaetous segments (an achaetous peristomium and a following achaetous segment with lateral organs) and 9 – 14 chaetigers with capillary chaetae. The last thoracic chaetiger may have capillary notochaetae and hooded neurohooks or capillary chaetae only in both rami. Abdomen with hooded hooks throughout or with one or more pre-anal segments with acicular spines in the notopodia. Pygidium expanded as a poorly or well developed anal plaque with a pair of ventral digitiform cirri. Anal plaque with embedded or protruding acicular spines.

#### **Key to all valid species of *Scyphoproctus***

- 1a.** Poorly developed anal plaque.....**2**
- 1b.** Well-developed developed anal plaque.....**3**
- 2a.** Digitated cirri; 8 – 11 pre-pygidial segments, with 10 spines each; two sets of spines on the plaque; thorax with nine chaetigers (Fig. 1B).....*S. armatus*
- 2b.** Filiform cirri; 6 – 8 pre-pygidial segments, with 4 – 8 spines each, increasing toward the plaque; thorax with 12 – 13 chaetigers (Fig. 1L).....*S. pseudoarmatus*

- 2c.** Heart-shaped cirri; one pre-pygidial segment, with two spines; thorax with 9 – 10 chaetigers; noto- and neuropodial hooded similar, with smooth hood (Fig. 1D).....*S. edmondsoni*
- 2d.** “W”-shaped cirri; four pre-pygidial segments, with three spines each; thorax with 9 – 10 chaetigers; noto- and neuropodial hooded different; neuropodial hooks with hood with fringed edge (Fig. 3E)..... *Scyphoproctus* sp. n. **C**
- 3a.** Anal plaque with the sets of acicular spines positioned marginally on the plaque, protruding or not from the edge.....**4**
- 3b.** Anal plaque with the sets of acicular spines in two transverse rows protruding midway in each side of the plaque, not reaching the edge.....**8**
- 4a.** Acicular spines embedded, not protruding from the edge; 11 – 12 chaetigers; anal plaque expanded in oblique plane with eight sets of spines each side; anus large; two long cirri (longer than plaque length) with a median membrane separating them (Fig. 1H).....*S. lumenalis*
- 4b.** Acicular spines visible, protruding from the edge of plaque.....**5**
- 5a.** Anal plaque without pre-pygidial segments.....**6**
- 5b.** Anal plaque with pre-pygidial segments.....**7**
- 6a.** Anal plaque funnel-shaped, with one chaetiger fused and 11 sets of acicular spines each side (4,4,3,3,3,2,2,2,1,1,1); two short and thin cirri without a median membrane separating them; median anus; thorax with 12 chaetigers (Fig. 1C).....*S. djiboutiensis*
- 6b.** Anal plaque circular, with one chaetiger fused and three sets of acicular spines each side (2,2,2); two long and thin cirri (longer than plaque length) with a median membrane separating them; median anus; thorax with 11 – 12 chaetigers (Fig. 1E).....*S. fasciculatus*
- 6c.** Anal plaque funnel-shaped, with 10 sets of acicular spines each side (16,11,6,4,3,4,3,1,1,1); two short and thin cirri without a median membrane separating them; large anus; thorax with 14 chaetigers (Fig. 1J).....*S. ornatus*

- 6d.** Anal plaque oval-shaped, with nine sets of acicular spines each side (3,3,2,2,1,1,1,1,1); two short and thin cirri without a median membrane separating them; small anus; thorax with 10 – 12 chaetigers (Figs. 5C and 6H).....*Scyphoproctus* sp. n. **B**
- 7a.** 2 – 6 pre-pygidial segments with one spine each; anal plaque funnel-shaped, with six sets of acicular spines each side (variable); two short and thick cirri without a median membrane separating them; small anus; thorax with 12 chaetigers (Fig. 1I).....*S. oculatus*
- 7b.** 2 pre-pygidial segments; anal plaque funnel-shaped, with 13 – 14 sets of acicular spines each side (6,4,4,3,3,2,2,1,1,1,1,1,1); two short and thin cirri without a median membrane separating them; small anus; thorax with 11 – 14 chaetigers (Fig. 1F).....*S. glabrus*
- 7c.** 2 pre-pygidial segments with 5 and 10 spines each, increasing toward the plaque; anal plaque circular and flattened, with nine sets of acicular spines each side (5,4,3,3,3,2,2,2,1); two short and thin cirri without a median membrane separating them; median anus; thorax with 12 chaetigers (Fig. 1G).....*S. guadalupensis*
- 7d.** 4 – 7 pre-pygidial segments with 2 – 7 spines each, increasing toward the plaque; anal plaque circular and flattened, with 5 – 7 sets of acicular spines each side (7,4,2,2,2,1,1); two short and thin cirri without a median membrane separating them; small anus; thorax with 12 chaetigers (Fig. 1K).....*S. platyproctus*
- 7d.** 3 pre-pygidial segments with 4 or 8 spines each, increasing toward the plaque; anal plaque oblique and flattened, with 7 sets of acicular spines each side (variable); two median and thin cirri without a median membrane separating them; median anus; thorax with 10 – 14 chaetigers (Fig. 1Q).....*S. variabilis*
- 8a.** 2 pre-pygidial segments with 3 and 7 spines each, increasing toward the plaque; anal plaque flattened dorsally, with two sets of acicular spines each side (5,3); two median and thin cirri without a median membrane separating them; small anus; thorax with 12 chaetigers (Fig. 1A).....*S. aciculatus*
- 8b.** 3 pre-pygidial segments with 3, 9, 7 spines; anal plaque flat, with two sets of acicular spines each side (3,4); two short and thin cirri with a median membrane separating them; median anus; thorax with 11 chaetigers (Fig. 1M).....*S. pullielloides*



- 8c.** 2 pre-pygidial segments; anal plaque oblique and flattened with lateral large flaps, 2 – 3 sets of acicular spines each side (variable); two short and thin cirri with a median membrane separating them; median anus; thorax with 11 – 12 chaetigers (Fig. 1N).....*S. somalus*
- 8d.** 3 pre-pygidial segments with 10 spines each; anal plaque flattened and slightly concave, four sets of acicular spines each side; two median and thin cirri with a median membrane separating them; large anus; thorax with 12 – 14 chaetigers (Fig. 1O).....*S. steinitzii*
- 8e.** 5 – 8 pre-pygidial segments with 1 – 13 spines each, increasing toward the plaque; anal plaque flattened with lateral flanges, three sets of acicular spines each side; two short and thin cirri with a long median membrane separating them; large anus; thorax with 11 – 12 chaetigers (Fig. 1P).....*S. towraiensis*
- 8f.** 5 pre-pygidial segments with 2 – 7 spines each, increasing toward the plaque; anal plaque flat with lateral flanges, 2 – 3 sets of acicular spines each side (2 – 5 spines each side); two median and thin cirri with a short median membrane separating them; small anus; thorax with 10 – 13 chaetigers (Figs. 2E and 3F, G).....*Scyphoproctus* sp. n. **A**

***Scyphoproctus* sp. n. A**

(Figures 2 – 4)

**Holotype:** Project “HABITATS”: Campos Basin (state of Rio de Janeiro). ZUEC POL 20596: 22°1'9"S – 40°31'55"W; sublittoral; 49 m deep; station HAB 17/FOZ 23; 24 Jul 2009; 1 spec.

**Additional material examined.** Project “REVIZEE” (states of Rio de Janeiro and São Paulo). ZUEC POL 6739: 25°11'S – 47°08'W; sublittoral; 157 m deep; station 6658; 16 Feb 1997; 5 specs. ZUEC POL 6746: 24°07'S – 44°42'W; sublittoral; 101 m deep; station 6669; 11 Jan 1998; 1 spec. ZUEC POL 6771: 23°20'S – 41°22'W; sublittoral; 110 m deep; station 6759; 28 Feb 1998; 1 spec. ZUEC POL 6772: 23°20'S – 41°22'W; sublittoral; 110 m deep; station 6759; 28 Feb 1998; 1 spec. ZUEC POL 6883: 23°20'S – 41°22'W; sublittoral; 110 m deep; station 6759; 28 Feb 1998; 2 specs. ZUEC POL 6887: 23°26'S – 41°15'W; sublittoral; 145 m deep; station 6762; 28 Feb 1998; 1 spec. ZUEC POL 6895: 23°08'S – 41°00'W; sublittoral; 100 m deep; station 6763; 01 Mar 1998; 1 spec. **Project “BIOTA/FAPESP - Araçá”:** São

**Sebastião Channel - Araçá Bay (state of São Paulo).** ZUEC POL 20523: 23°48'S – 45°24'W; tidal flat; station 7(3); 24 Nov 2011; 4 specs. ZUEC POL 20524: 23°48'S – 45°24'W; tidal flat; station 4(2)A; 26 Sep 2011; 1 spec. ZUEC POL 20525: 23°48'S – 45°24'W; tidal flat; station 4(1)A; 26 Sep 2011; 5 specs. ZUEC POL 20526: 23°48'S – 45°24'W; tidal flat; station 37(2)A; 05 Feb 2011; 1 spec. ZUEC POL 20527: 23°48'S – 45°24'W; tidal flat; station 39(2)A; 05 Feb 2011; 1 spec. ZUEC POL 20528: 23°48'S – 45°24'W; tidal flat; station 4(3)A; 26 Sep 2011; 2 specs. ZUEC POL 20529: 23°48'S – 45°24'W; tidal flat; station 38(1)A; 05 Feb 2011; 7 specs. ZUEC POL 20530: 23°48'S – 45°24'W; tidal flat; station 39(3)A; 05 Feb 2011; 1 spec. ZUEC POL 20531: 23°48'S – 45°24'W; tidal flat; station 20(2)A; 29 Oct 2011; 1 spec. ZUEC POL 20532: 23°48'S – 45°24'W; tidal flat; station 38(2)A; 05 Feb 2011; 1 spec. ZUEC POL 20533: 23°48'S – 45°24'W; tidal flat; station 38(3)A; 05 Feb 2011; 3 specs. ZUEC POL 20534: 23°48'S – 45°24'W; tidal flat; station 39(1)B; 05 Feb 2011; 1 spec. ZUEC POL 20535: 23°48'S – 45°24'W; tidal flat; station 39(1)A; 05 Feb 2011; 17 specs. **Project “BioPol-NE”: Cabo Branco Beach (state of Paraíba).** ZUEC POL 20536: 7°08'S – 34°47'W; rhodoliths; 01 Feb 2010; 1 spec. ZUEC POL 20538: 7°08'S – 34°47'W; rhodoliths; 08 Feb 2009; 1 spec. **Jacumã Beach (State of Paraíba).** ZUEC POL 20537: 7°14'S – 34°47'W; 29 Jan 2010; 1 spec. **Project “Oil Spill”: São Sebastião Channel - São Francisco Beach (state of São Paulo).** ZUEC POL 20539: 23°44'54"S – 45°24'34"W; sandy beach; station 45; 23 Jun 2013; 2 specs. **Project “HABITATS”: Campos Basin (state of Espírito Santo).** **Continental Shelf:** ZUEC POL 20540: 22°52'1"S – 40°57'28"W; sublittoral; 90 m deep; station HAB 16/C04; 03 Jul 2009; 2 specs. ZUEC POL 20541: 22°52'1"S – 40°57'28"W; sublittoral; 92 m deep; station HAB 11/C04; 22 Feb 2009; 1 spec. ZUEC POL 20542: 21°42'37"S – 40°8'59"W; sublittoral; 147 m deep; station HAB 16/H05; 07 Jul 2009; 6 specs. ZUEC POL 20543: 21°23'2"S – 40°15'9"W; sublittoral; 147 m deep; station HAB 13/I05; 06 Mar 2009; 5 specs. ZUEC POL 20544: 22°17'42"S – 40°26'59"W; sublittoral; 103 m deep; station HAB 11/E04; 23 Feb 2009; 4 specs. ZUEC POL 20545: 23°12'8"S – 40°59'35"W; sublittoral; 142 m deep; station HAB 16/B05; 02 Jul 2009; 1 spec. ZUEC POL 20546: 21°23'37"S – 40°15'38"W; sublittoral; 88 m deep; station HAB 17/I03; 21 Jul 2009; 2 specs. ZUEC POL 20547: 22°17'42"S – 40°26'59"W; sublittoral; 103 m deep; station HAB 16/E04; 04 Jul 2009; 5 specs. ZUEC POL 20548: 21°9'10"S – 40°16'7"W; sublittoral; 101 m deep; station HAB 13/I04; 07 Mar 2009; 1 spec. ZUEC POL 20549: 22°3'45"S – 40°9'59"W; sublittoral; 75 m deep; station HAB 11/G03; 25 Feb 2009; 2 specs. ZUEC POL 20550: 21°9'9"S – 40°16'7"W; sublittoral;

104 m deep; station HAB 17/I04; 21 Jul 2009; 8 specs. ZUEC POL 20551: 22°12'38"S – 40°13'18"W; sublittoral; 99 m deep; station HAB 16/F04; 05 Jul 2009; 6 specs. ZUEC POL 20552: 22°12'37"S – 40°13'18"W; sublittoral; 99 m deep; station HAB 11/F04; 24 Feb 2009; 7 specs. ZUEC POL 20553: 21°43'10"S – 40°11'30"W; sublittoral; 72 m deep; station HAB 16/H03; 08 Jul 2009; 3 specs. ZUEC POL 20554: 21°42'53"S – 40°10'16"W; sublittoral; 98 m deep; station HAB 13/H04; 09 Mar 2009; 1 spec. ZUEC POL 20555: 22°19'31"S – 40°37'19"W; sublittoral; 73 m deep; station HAB 11/D03; 23 Feb 2009; 17 specs. ZUEC POL 20556: 22°46'55"S – 41°3'33"W; sublittoral; 78 m deep; station HAB 11/C03; 22 Feb 2009; 3 specs. ZUEC POL 20557: 23°10'5"S – 41°3'6"W; sublittoral; 107 m deep; station HAB 16/B04; 02 Jul 2009; 2 specs. ZUEC POL 20558: 22°7'43"S – 40°18'46"W; sublittoral; 73 m deep; station HAB 11/F03; 24 Feb 2009; 1 spec. ZUEC POL 20559: 22°12'53"S – 40°51'12"W; sublittoral; 52 m deep; station HAB 11/D02; 26 Feb 2009; 1 spec. ZUEC POL 20560: 21°42'37"S – 40°8'59"W; sublittoral; 147 m deep; station HAB 13/H05; 09 Mar 2009; 2 specs. ZUEC POL 20561: 21°22'59"S – 40°19'41"W; sublittoral; 52 m deep; station HAB 13/I02; 05 Mar 2009; 2 specs. ZUEC POL 20562: 22°37'35"S – 41°21'51"W; sublittoral; 53 m deep; station HAB 11/B02; 27 Feb 2009; 1 spec. ZUEC POL 20563: 22°19'31"S – 40°37'19"W; sublittoral; 73 m deep; station HAB 16/D03; 04 Jul 2009; 12 specs. ZUEC POL 20564: 21°42'53"S – 40°10'15"W; sublittoral; 98 m deep; station HAB 16/H04; 07 Jul 2009; 1 spec. ZUEC POL 20565: 21°22'58"S – 40°19'41"W; sublittoral; 53 m deep; station HAB 17/I02; 21 Jul 2009; 1 spec. ZUEC POL 20566: 22°45'49"S – 41°45'33"W; sublittoral; 53 m deep; station HAB 17/B02; 16 Jul 2009; 2 specs. ZUEC POL 20567: 22°59'47"S – 41°21'7"W; sublittoral; 77 m deep; station HAB 11/B03; 21 Feb 2009; 1 spec. ZUEC POL 20568: 22°57'28"S – 40°50'30"W; sublittoral; 143 m deep; station HAB 16/C05; 03 Jul 2009; 1 spec. ZUEC POL 20569: 22°3'45"S – 40°9'58"W; sublittoral; 75 m deep; station HAB 16/G03; 06 Jul 2009; 1 spec. **Paraíba do Sul river mouth.** ZUEC POL 20592: 22°12'31"S – 40°14'8"W; sublittoral; 97 m deep; station HAB 17/FOZ 43; 24 Jul 2009; 3 specs. ZUEC POL 20593: 22°1'10"S – 40°31'53"W; sublittoral; 49 m deep; station HAB 13/FOZ 23; 12 Mar 2009; 2 specs. ZUEC POL 20594: 21°40'25"S – 40°58'26"W; sublittoral; 18 m deep; station HAB 17/FOZ 5; 19 Jul 2009; 2 specs. ZUEC POL 20595: 21°21'21"S – 40°52'9"W; sublittoral; 20 m deep; station HAB 17/FOZ 2; 23 Jul 2009; 1 spec. ZUEC POL 20597: 21°17'31"S – 40°48'21"W; sublittoral; 24 m deep; station HAB 13/FOZ 14; 08 Mar 2009; 1 spec. ZUEC POL 20598: 22°1'9"S – 40°31'55"W; sublittoral; 49 m deep; station HAB

17/FOZ 23; 24 Jul 2009; 2 specs. ZUEC POL 20599: 21°45'13"S – 40°14'7"W; sublittoral; 67 m deep; station HAB 17/FOZ 41; 24 Jul 2009; 5 specs.

**Description.** Complete specimens ranged from 0.2 – 0.8 mm wide, 2.5 – 22 mm long and 15 – 58 chaetigers; incomplete specimens ranged from 0.2 – 1.5 mm wide. Body rounded; thorax almost the same width than abdomen (Figs. 2A, B and 3A); abdomen the same width toward the pygidium. Color in alcohol whitish. Prostomium robust, wider than longer at the basis, rounded on anterior end (Fig. 2A, H); nuchal organs not observed; eyespots present as a pair of oblique pigmented area (Fig. 2A). Proboscis globular-shaped, finely papillated. Peristomium achaetous, longer than the other thoracic segments (Figs. 2A, B and 3A). Thorax with 12 – 15 segments, including the peristomium, one additional achaetous segment, and 10 – 13 chaetigers. Thorax smooth; chaetigers strongly biannulate and rectangular; incomplete inter-segmental groove traversing the achaetous segment and first chaetiger (Figs. 2A, B, H and 3A). First chaetiger biramous. Noto- and neuropodial lobes well separated; notopodia inserted dorsally and neuropodia ventrally (Fig. 3A). Lateral organs onwards achaetous segment, more conspicuous on larger specimens. Adult specimens with bilimbate capillaries in notopodia and neuropodia of chaetigers 1 – 10/13. Transition between thorax and abdomen subtle, marked mainly by chaetal change (Figs. 2A and 3A). Abdominal chaetigers with hooded hooks throughout. Abdominal notopodia and neuropodia with poorly developed tori pads; first 1 – 3 chaetigers (size dependent) with notopodial lobes completely together, next chaetigers with lobes well separated throughout the abdomen (Figs. 2C; 3A and 4A, C, D); neuropodial lobes separated, with nearly the same distance throughout the abdomen (Figs. 2D and 4B, C, D). First notopodia with 3 – 42 hooks per fascicle, decreasing posteriorwards; first neuropodia with 3 – 36 hooks per fascicle, maintaining the number of hooks until the middle abdomen. Anterior notopodial hooks with long and rounded main fang, right angle with the shaft, surmounted by approximately 16 teeth arranged in four rows (3 – 4 – 5 – 4); thick hood with smooth edge, regular aperture (Fig. 3B). Posterior notopodial hooks with long and pointed main fang, right angle with the shaft, surmounted by more than 30 teeth arranged in six rows (intercalated pattern among teeth of the tori); thin hood with smooth edge, irregular aperture (narrow at the top and large at the basis) (Fig. 3E). Anterior neuropodial hooks (first two chaetigers) with long and pointed main fang, right angle with the shaft, surmounted by approximately 20 teeth arranged in four rows; thick hood with smooth edge, regular aperture (Fig. 3F). Posterior neuropodial hooks (from third chaetiger) with large and robust main fang,

right angle with the shaft, surmounted by approximately 40 teeth arranged in six rows; large thin hood with robust fringed edge, regular aperture (Fig. 3G). Branchiae absent. Five pre-pygidial segments with rounded lateral lobes and notopodial spines; 2 – 7 notopodial spines and neuropodial hooks (size dependent); pygidium with two chaetiger fused with anal plaque; plaque flat with elevated edges, 2 – 3 sets of spines present (2 – 5 spines each side); small anus (5% of plaque length) near the inferior margin, not extending to edge of the plaque; two median thin cirri (two-thirds the plaque length), with a median membrane (Figs. 2F, G; 3C, D and 4E).

**Methyl green staining pattern.** Tip of prostomium staining darkly (Fig. 2H). Dorsal region of peristomium, achaetous segment and chaetiger 1 with a large continuous dark speckle (Fig. 2H). Thoracic chaetigers staining uniformly green (Fig. 2B). In abdominal region, first 2 – 3 chaetigers staining entirely; the next few chaetigers staining dorsally with a dark band in the line of the tori and around them; afterwards staining is restricted to parapodial tori, but more intense on notopodia (Figs. 2B, E, I). Dark little speckles around abdominal lateral organs (Fig. 2E).

**Remarks.** *Scyphoproctus* A sp. n. belongs to a group of species with the sets of acicular spines in two transverse rows protruding at midlength in each side of the plaque, not reaching the edge, which includes *S. aciculatus*, *S. pullielloides*, *S. somalus*, *S. steinitzii* and *S. towraiensis*.

*Scyphoproctus aciculatus*, *S. pullielloides*, *S. somalus* and *S. steinitzii* differ from *Scyphoproctus* sp. n. A by the number of pre-pygidial segments, which is three in *S. aciculatus*, *S. pullielloides* and *S. steinitzii*, two in *S. somalus*, and five in *Scyphoproctus* sp. n. A. *Scyphoproctus aciculatus* and *S. steinitzii* are also different from the new species by the absence of a median membrane separating the cirri and by the number of rows of spines on the plaque, two in *S. aciculatus* and four in *S. steinitzii*, in contrast with 2 – 3 of *Scyphoproctus* sp. n. A.

*Scyphoproctus* sp. n. A shares several similarities with *S. towraiensis* such as the number of pre-pygidial segments, incomplete inter-segmental groove traversing the achaetous segment and first chaetiger, and four types of hooded hooks along the abdomen. However, the latter has three segments fused with the plaque and the former has two; *S. towraiensis* presents up to 13 acicular spines on pre-pygidial segment while *Scyphoproctus* sp. n. A has up to seven; the number of chaetigers in *Scyphoproctus* sp. n. A is greater (10 – 13) than in *S.*

*towraiensis* (11 – 12); additionally, besides the equal number of types of hooded hooks, they vary between both species.

**Habitat.** From intertidal to sublittoral regions (0 – 157 m); in sand, mud and rhodolits.

**Type locality.** Rio de Janeiro, Brazil (South Atlantic Ocean).

**Distribution.** South Atlantic Ocean: Brazil (states of Espírito Santo, Paraíba, Rio de Janeiro and São Paulo).

### ***Scyphoproctus* sp. n. B**

(Figures 5 – 7)

**Holotype: Project “FDP – Fauna Beach”:** São Sebastião Channel (state of São Paulo). ZUEC POL 20522: 23°44'54"S – 45°24'34"W; sandy beach; station 445A; 04 Oct 1995; 1 spec.

**Paratypes 1 – 7: Project “Oil Spill”:** São Sebastião Channel (state of São Paulo). São Paulo, São Francisco Beach: ZUEC POL 20513: 23°44'54"S – 45°24'34"W; sandy beach; station 47; 23 Jun 2013; 7 specs.

**Additional material examined. Project “FDP – Fauna Beach”:** São Sebastião Channel – Engenho D’Água Beach (state of São Paulo). ZUEC POL 1779: 23°47'49"S – 45°21'56"W; sandy beach; station 2807B; 25 Nov 1996; 1 spec. ZUEC POL 1854: 23°47'49"S – 45°21'56"W; sandy beach; station 1883B; 20 May 1996; 1 spec. ZUEC POL 1855: 23°47'49"S – 45°21'56"W; sandy beach; station 1881A; 20 May 1996; 2 specs. ZUEC POL 1935: 23°47'49"S – 45°21'56"W; sandy beach; station 3199B; 08 Apr 1997; 2 specs. ZUEC POL 1936: 23°47'49"S – 45°21'56"W; sandy beach; station 3196A; 08 Apr 1996; 6 specs. ZUEC POL 1954: 23°47'49"S – 45°21'56"W; sandy beach; station 3199B; 02 Jul 1996; 1 spec. **São Francisco Beach (state of São Paulo).** ZUEC POL 20521: 23°44'54"S – 45°24'34"W; sandy beach; station 445A; 04 Dec 1995; 3 specs. **Project “BIOPORE”:** Caraguatatuba Bay - Camaroeiro Beach (state of São Paulo). ZUEC POL 20520: 23°37'38"S – 45°23'50"W; mussel bed; station 16A; 25 May 2006; 1 spec. **Project “BioPol-NE”:** Cabo Branco Beach (state of Paraíba). ZUEC POL 20518: 7°08'S – 34°47'W;



rhodoliths; 08 Feb 2009; 5 specs. ZUEC POL 20519: 7°08'S – 34°47'W; rhodoliths; 01 Feb 2010; 2 specs. **Project “AMBES”: Espírito Santo Basin – Continental Shelf (state of Espírito Santo).** ZUEC POL 20507: 18°40'55,3"S – 38°55'41,48"W; sublittoral; 44 m deep; station AMB 7/G 03/R2; 17 Jan 2012; 2 specs. ZUEC POL 20508: 21°2'45,81"S – 40°32'29,21"W; sublittoral; 20 m deep; station AMB 7/A 01/R3; 22 Jan 2012; 1 spec. ZUEC POL 20509: 18°40'55,3"S – 38°55'41,48"W; sublittoral; 34 m deep; station AMB 7/E 02/R1; 02 Dec 2011; 1 spec. **Project “Oil Spill”: São Sebastião Channel - São Francisco Beach (state of São Paulo).** ZUEC POL 20510: 23°44'54"S – 45°24'34"W; sandy beach; station 45; 26 Jun 2013; 3 specs. ZUEC POL 20511: 23°44'54"S – 45°24'34"W; sandy beach; station 72; 26 Jun 2013; 1 spec. ZUEC POL 20512: 23°44'54"S – 45°24'34"W; sandy beach; station 71; 26 Jun 2013; 9 specs. ZUEC POL 20514: 23°44'54"S – 45°24'34"W; sandy beach; station 20P; 11 Apr 2013; 15 specs. ZUEC POL 20515: 23°44'54"S – 45°24'34"W; sandy beach; station 5G; 11 Apr 2013; 2 specs. ZUEC POL 20516: 23°44'54"S – 45°24'34"W; sandy beach; station 43; 26 Jun 2013; 7 specs. **Caraguatatuba - Enseada Beach (state of São Paulo).** ZUEC POL 20517: 23°43'40"S – 45°25'10"W; sandy beach; station 47P; 11 Apr 2013; 6 specs. **Project “BIOTA/FAPESP - Araçá”: São Sebastião Channel - Araçá Bay (state of São Paulo).** ZUEC POL 20504: 23°48'56,4"S – 45°24'22,2"W; tidal flat; station 10(3)B; 14 Jun 2011; 1 spec. ZUEC POL 20505: 23°48'55,9"S – 45°23'58,0"W; shallow subtidal; 8.6 m deep; station 29(1)Mc; 10 Oct 2013; 1 spec. ZUEC POL 20506: 23°48'47,5"S – 45°24'25,1"W; tidal flat; station 22(1)A; 15 Jun 2011; 1 spec.

**Description.** Size range of material examined (incomplete specimens) 0.30 – 1.5 mm wide and 32 – 66 chaetigers. Body robust; similar width between thorax and abdomen; slightly narrowing toward the pygidium. Color in alcohol whitish. Prostomium robust, two times longer than wider, rounded on anterior end, generally hidden by the peristomium (Figs. 5A; 6A and 7A); nuchal organs not observed; eyespots present as a pair of oval postero-lateral densely pigmented areas. Proboscis globular-shaped, with several robust papillae (Figs. 5B and 6C). Peristomium achaetous, 1.5x longer than thoracic segments (Figs. 5A; 6A and 7A). Thorax with 12 – 14 segments, including the peristomium, one additional achaetous segment and 10 – 12 chaetigers (Figs. 5A; 6A and 7A). Peristomium and the next achaetous segment weakly rugose (Fig. 5A). Thorax with chaetigers distinctly biannulate, more evident after chaetigers 5 – 6 (Figs. 6A and 7A); complete inter-segmental groove traversing the achaetous segment (Figs. 5A and 6A). First chaetiger biramous. Notopodial lobes inserted dorso-

laterally and neuropodial lobes laterally. Lateral organs present since the achaetous segment and throughout the thorax, between noto- and neuropodia, closer to notopodia (Figs. 5A; 6D and 7A). Adult specimens with bilimbate capillaries in notopodia and neuropodia of chaetigers 1 – 10/12. Transition between thorax and abdomen inconspicuous, marked only by change of chaetae and methyl green staining pattern (Figs. 5A and 6A, B). Abdominal chaetigers with hooded hooks throughout. Abdominal noto- and neuropodia with glandular tori pads separated by the same distance throughout (Figs. 5D, E and 6E, F). First notopodial chaetigers with 3 – 20 hooks per fascicle, reducing toward the posterior end; first neuropodial chaetigers with 6 – 20 hooks per fascicle, increasing toward mid abdomen, and reducing toward the posterior end. Notopodial hooks with a thin and rounded on the tip main fang, right angle with the shaft, surmounted by 12 teeth arranged in four rows (3 – 3 – 3 – 3); large and smooth hood (Fig. 7B). Neuropodial hooks with a large and robust main fang, right angle with the shaft, surmounted by 22 teeth arranged in three rows (4 – 6 – 12); large hood with a serrate edge (Fig. 7C). Branchiae absent. Pre-pygidial segments with noto- and neuropodial hooks; pygidium with one chaetiger fused with anal plaque; plaque flat, oval shaped, nine set of acicular spines protruding from the edge of the plaque (3–3–2–2–1–1–1–1–1 acicular spines on each side); small anus (5% of plaque length) near the inferior margin, not extending to edge of the plaque; two short thin fused cirri (one-third the plaque length), without median membrane (Figs. 5C, F and 6H, I).

**Methyl green staining pattern.** Thoracic segments staining uniformly darker than the abdomen; final thoracic chaetigers with a fine dark band on the inter- and intra-segmental grooves; abdominal chaetigers staining uniformly light green (Fig. 6B).

**Remarks.** *Scyphoproctus* B sp. n. belongs to a group of species with the sets of acicular spines positioned marginally on the plaque, protruding or not from the edge, which includes *Scyphoproctus djiboutiensis*, *S. fasciculatus*, *S. glabrus*, *S. guadalupensis*, *S. lumenalis*, *S. oculatus*, *S. ornatus*, *S. platyproctus* and *S. variabilis*.

*Scyphoproctus djiboutiensis*, *S. fasciculatus*, *S. glabrus*, *S. guadalupensis* and *S. ornatus* are similar to *Scyphoproctus* sp. n. B in having only one chaetiger fused with the anal plaque. However, *S. glabrus* and *S. guadalupensis* differ from *Scyphoproctus* sp. n. B in having two pre-pygidial segments with notopodia bearing acicular spines, while the latter species has hooded hooks on both noto- and neuropodia. The differences between *Scyphoproctus* sp. n. B and *S. glabrus* are also related to the number of sets of acicular spines

of the anal plaque, which is nine in the former and fourteen in the latter, and the features of the abdominal hooded hooks. *Scyphoproctus glabrus* has both noto- and neuropodial hooded hooks similar, with 10 – 15 teeth above main fang arranged in three rows, while *Scyphoproctus* sp. n. B has notopodial hooded hooks with 12 teeth arranged in four rows and neuropodial hooded hooks with 22 teeth arranged in three rows.

*Scyphoproctus fasciculatus* and *S. ornatus* differ from *Scyphoproctus* sp. n. B by the number of sets of acicular spines of the anal plaque, number of acicular spines, length of anus and features of the cirri. *Scyphoproctus fasciculatus* presents anal plaque with three set of acicular spines with two spines each, anus with moderate length (18% of plaque length) and cirri longer than the plaque with a median membrane separating them. *Scyphoproctus ornatus* presents anal plaque with ten sets of acicular spines with several spines each (16-11-6-4-3-4-3-1-1-1) and large anus (25% of plaque length).

*Scyphoproctus oculatus*, *S. platyproctus* and *S. variabilis* differ from *Scyphoproctus* sp. n. B by the presence of pre-pygidial segments with acicular spines, different from the latter species, which has none. *Scyphoproctus oculatus* has 2 – 6, *S. platyproctus* has 4 – 7 and *S. variabilis* has two. Although *S. lumenalis* also lacks pre-pygidial segments with acicular spines, it differs from *Scyphoproctus* B sp. n. because the spines of the plaque are embedded and by the presence of two long cirri (longer than plaque length) with a median membrane separating them, in contrast with *Scyphoproctus* sp. n. B, which has protruding spines, and short cirri without a median membrane.

Although *Scyphoproctus djiboutiensis* also has thoracic region with 14 segments (12 chaetigers) and short cirri without a median membrane, the species differs from *Scyphoproctus* sp. n. B by the number of sets of acicular spines, which is 11, lack of eyespots, proboscis without papillae, thorax smooth, absence of lateral organs and noto- and neuropodial hooded hooks similar.

**Habitat.** From intertidal to sublittoral regions (0 – 44 m); in sand, mussel bed and rhodolits.

**Type locality.** São Francisco beach, São Paulo, Brazil (South Atlantic Ocean).

**Distribution.** South Atlantic Ocean: Brazil (states of Espírito Santo, Paraíba and São Paulo).

### *Scyphoproctus* sp. n. C

(Figures 8 – 10)

**Holotype: Project “BIOTA/FAPESP - Araçá”:** São Sebastião Channel - Araçá Bay (state of São Paulo). ZUEC POL 20591: 23°48'S – 45°24'W; rocky shore; 15 Feb 2013; 1 spec.

**Paratypes 1 – 9: Project “BIOTA/FAPESP - Araçá”:** São Sebastião Channel - Araçá Bay (state of São Paulo). ZUEC POL 20574: 23°48'S – 45°24'W; rocky shore; 16 May 2014; 9 specs.

**Additional material examined. Project “BIOTA/FAPESP - Araçá”:** São Sebastião Channel - Araçá Bay (state of São Paulo). ZUEC POL 20571: 23°48'S – 45°24'W; rocky shore; 19 Mar 2015; 2 specs. ZUEC POL 20572: 23°48'S – 45°24'W; rocky shore; 15 Feb 2013; 7 specs. ZUEC POL 20573: 23°48'S – 45°24'W; tidal flat; station 4(3)A; 26 Sep 2011; 1 spec. ZUEC POL 20575: 23°48'S – 45°24'W; rocky shore; 10 Nov 2013; 1 spec. ZUEC POL 20576: 23°48'45,8"S – 45°24'28,9"W; tidal flat; station 152(1)G; 23 Feb 2013; 1 spec. ZUEC POL 20577: 23°48'S – 45°24'W; tidal flat; station 20(2)A; 29 Oct 2011; 1 spec. ZUEC POL 20578: 23°48'39,4"S – 45°24'26,2"W; tidal flat; station 3(2)Pr; 12 Oct 2012; 1 spec. ZUEC POL 20579: 23°48'55,9"S – 45°23'58,0"W; tidal flat; station 66(2)Pr; 08 Feb 2012; 1 spec. **Project “Oil Spill”:** São Sebastião Channel - São Francisco Beach (state of São Paulo). ZUEC POL 20580: 23°44'54"S – 45°24'34"W; sandy beach; station 45; 23 Jun 2013; 1 spec. ZUEC POL 20581: 23°44'54"S – 45°24'34"W; sandy beach; station 72; 23 Jun 2013; 3 specs. ZUEC POL 20582: 23°44'54"S – 45°24'34"W; sandy beach; station 48; 23 Jun 2013; 2 specs. ZUEC POL 20583: 23°44'54"S – 45°24'34"W; sandy beach; station 20P; 11 Apr 2013; 6 specs. ZUEC POL 20584: 23°44'54"S – 45°24'34"W; sandy beach; station 5G; 11 Apr 2013; 2 specs. ZUEC POL 20585: 23°44'54"S – 45°24'34"W; sandy beach; station 43; 23 Jun 2013; 3 specs. **Caraguatatuba - Enseada Beach (state of São Paulo).** ZUEC POL 20586: 23°43'40"S – 45°25'10"W; sandy beach; station 47P; 12 Apr 2013; 3 specs. **Project “BioPol-NE”:** Cabo Branco Beach (state of Paraíba). ZUEC POL 20587: 7°08'S – 34°47'W; rhodoliths; 01 Feb 2010; 3 specs. ZUEC POL 20588: 7°08'S – 34°47'W; rhodoliths; 08 Feb 2009; 1 spec. **Jacumã Beach (Sate of Paraíba).** ZUEC POL 20589: 7°14'S – 34°47'W; 29 Jan 2010; 1 spec.

**Description.** Complete specimens ranged from 0.3 – 1.1 mm wide, 2.5 – 15 mm long and 24 – 79 chaetigers; incomplete specimens ranged from 0.3 – 2.2 mm wide. Body rounded; thorax wider than abdomen; abdomen slightly narrowing toward the pygidium (Fig. 9A). Color in alcohol whitish. Prostomium rectangular, 0.5 times longer than wider, rounded on anterior end (Figs. 8A; 9B, C and 10A); nuchal organs not observed; eyespots present as a pair of densely pigmented red area, forming an inverted “U” (Figs. 8A and 9C). Proboscis globular-shaped, with several robust papillae (Fig. 9C). Peristomium achaetous, longer than achaetous segment (Figs. 8A; 9B and 10A). Thorax with 11 – 12 segments, including the peristomium, one additional achaetous segment, and 9 – 10 chaetigers. Thorax smooth with chaetigers 1 – 3 weakly biannulate and next thoracic chaetigers strongly biannulate; complete inter-segmental groove traversing the achaetous segment (Figs. 8A and 10A). First chaetiger biramous. Parapodial lobes well separated; notopodia inserted dorso-laterally and neuropodia laterally. Lateral organs onwards achaetous segment, more conspicuous on larger specimens. Adult specimens with bilimbate capillaries in notopodia and neuropodia of chaetigers 1 – 9/10. Transition between thorax and abdomen subtle, marked mainly by chaetal change (Figs. 8A, 9A and 10A). Abdominal chaetigers with hooded hooks throughout. Abdominal notopodia and neuropodia with inflated tori pads well separated, the same distance through the abdomen (Fig. 9D, F). First notopodial chaetiger with 2 – 40 hooks per fascicle; first neuropodial chaetiger with 5 – 43 hooks per fascicle; in both rami the number of hooks increases on next two to three chaetigers and afterwards reduces toward the posterior end. Notopodial hooks with rounded and large main fang, right angle with the shaft, surmounted by more than 20 teeth arranged in 4 – 5 rows (3 – 4 basally, intercalated among the teeth of the tori; remaining teeth smaller and with diversified arrangement); thick hood with smooth edge (Figs. 9C, D). Neuropodial hooks with pointed and large main fang, right angle with the shaft, surmounted by 15 – 16 teeth arranged in three rows (3 – 4 basally, intercalated among the teeth of the tori; 5 – 6 median; 6 – 7 apically); thin hood with a slightly fringed edge (little and pointed fringes) (Fig. 9E). Branchiae absent. Four pre-pygidial segments with three notopodial spines and neuropodial hooks; anal plaque poorly developed, “w”-shaped, with one set of spines (1 spine on each side); large anal aperture between the two cirri; two short thick cirri (Fig. 10B).

**Methyl green staining pattern.** Chaetigers 1 and 2 with a line of large dark speckles around the segment, spaced equally from each other; chaetigers 6 – 9 staining with little dark

speckles; first two abdominal chaetigers staining darkly around the noto- and neuro tori (Fig. 9F).

**Remarks.** *Scyphoproctus* sp. n. C belongs to a group of species with a poorly developed anal plaque, which includes *S. armatus*, *S. edmondsoni*, and *S. pseudoarmatus*. *Scyphoproctus armatus* and *S. pseudoarmatus* differ from *Scyphoproctus* sp. n. C by the number of thoracic chaetigers, number of pre-pygidial segments with acicular spines, and the shape of anal cirri. *Scyphoproctus armatus* has nine thoracic chaetigers, *S. pseudoarmatus* has 12 – 13 and *Scyphoproctus* sp. n. C, has 9 – 10. The number of pre-pygidial segments with acicular spines in *S. armatus* is 8 – 11, in *S. pseudoarmatus*, 6 – 8 and in *Scyphoproctus* sp. n. C, four pre-pygidial segments. Anal cirri are digitate in *S. armatus*, filiform in *S. pseudoarmatus* and “w”-shaped in *Scyphoproctus* sp. n. C.

*Scyphoproctus edmondsoni* also has 9 – 10 thoracic chaetigers, abdominal noto- and neuropodia with well separated glandular tori pads, and inflated anal cirri, but it differs from *Scyphoproctus* sp. n. C by the number of pre-pygidial segments with acicular spines and by the hooded hooks, such as number and arrangement of teeth and format and thickness of the hood. *Scyphoproctus* sp. n. C has four pre-pygidial segments with acicular spines, while *S. edmondsoni* has only one. Hooded hooks in *Scyphoproctus* sp. n. C are different between noto- and neuropodia. Notopodial hooks have more than 20 teeth arranged in 4 – 5 rows and thick hood with smooth edge; neuropodial hooks have 15 – 16 teeth arranged in three rows and thin hood with a slightly fringed edge (little and pointed fringes). On the other hand, hooded hooks of *S. edmondsoni* apparently are similar between noto- and neuropodia, with several teeth arranged in 3 – 4 rows, and with a thick and smooth hood.

**Habitat.** Intertidal region, in mud, rhodolits and rocky shore.

**Type locality.** Aracá Bay, São Paulo, Brazil (South Atlantic Ocean).

**Distribution.** South Atlantic Ocean: Brazil (states of Paraíba and São Paulo).

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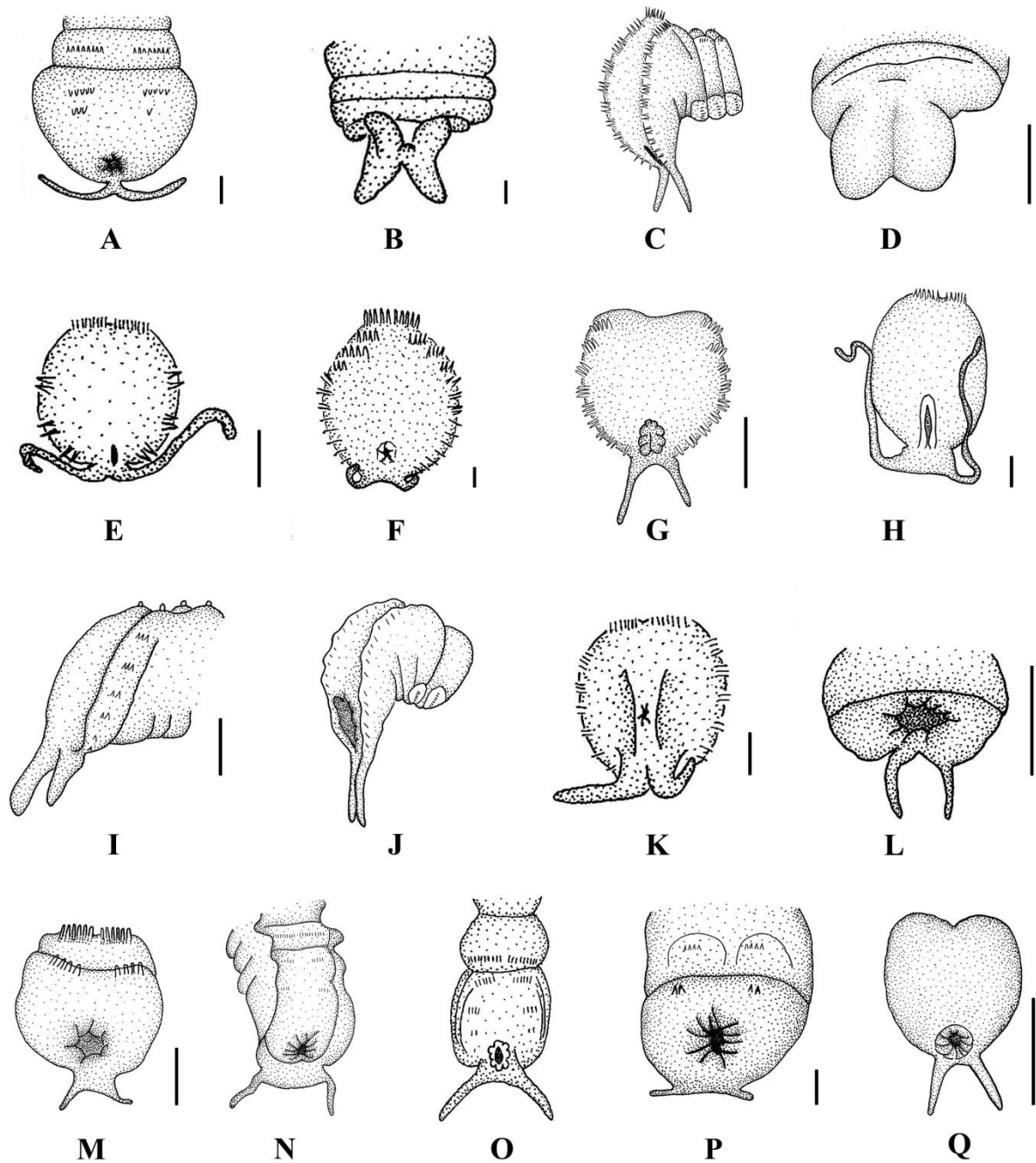
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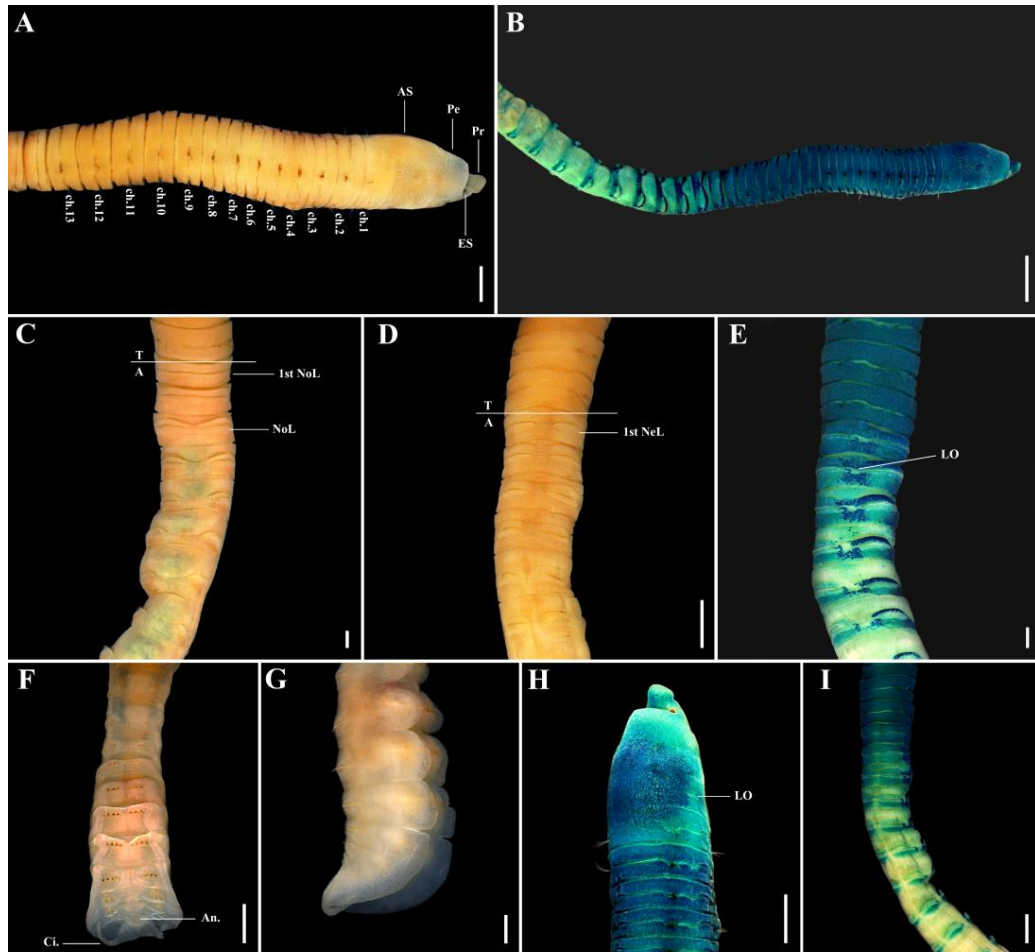
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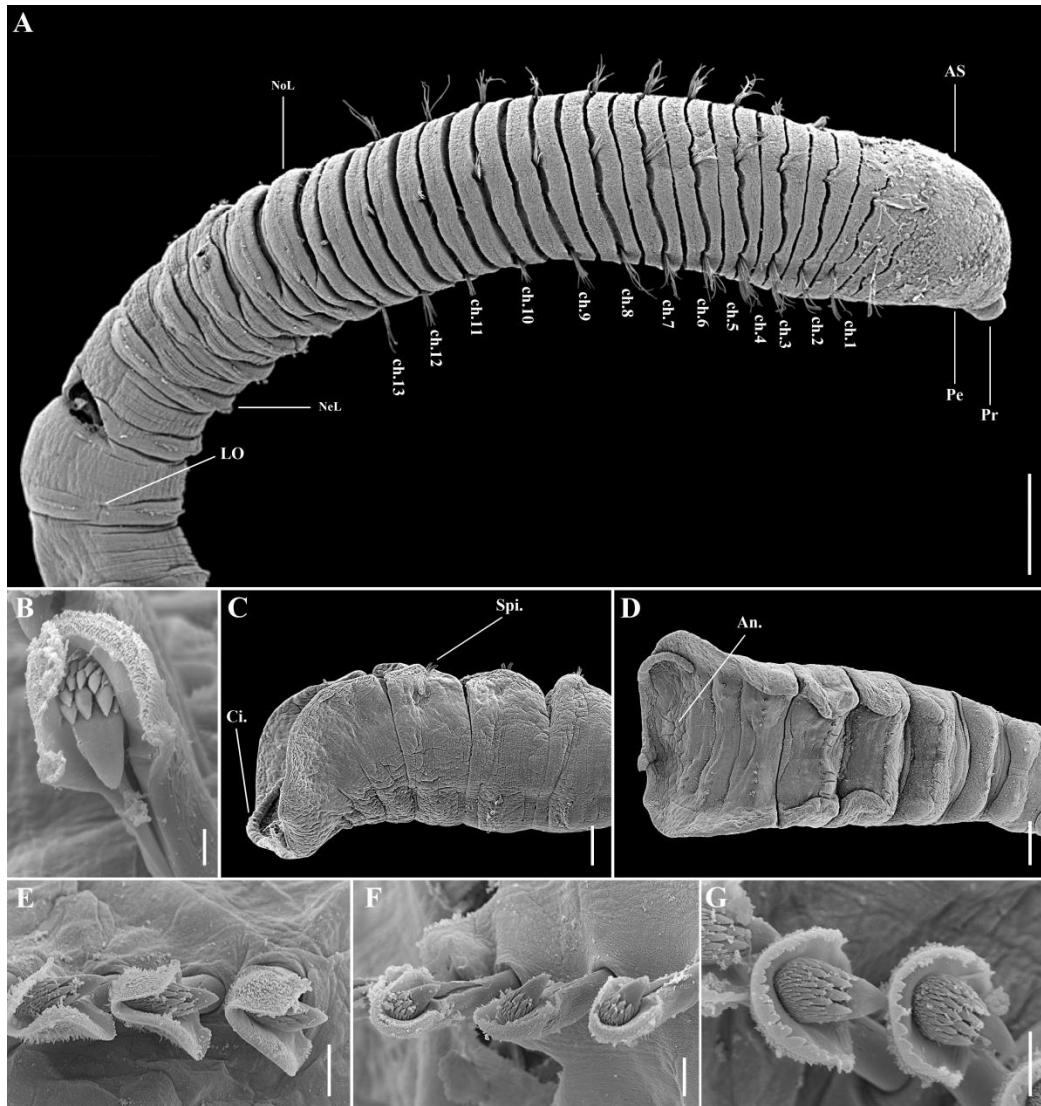
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**Fig. 1.** *Scyphoproctus* species. Anal plaques. A: *S. aciculatus*. B: *S. armatus*. C: *S. djiboutiensis*. D: *S. edmondsoni*. E: *S. fasciculatus*. F: *S. glabrus*. G: *S. guadalupensis*. H: *S. lumenalis*. I: *S. oculatus*. J: *S. ornatus*. K: *S. platyproctus*. L: *S. pseudoarmatus*. M: *S. pullielloides*. N: *S. somalus*. O: *S. steinitzii*. P: *S. towraiensis*. Q: *S. variabilis*. Scales bars: A, E, F, H, K, P, 0.2 mm; B, 0.35 mm; D, 0.125 mm; G, Q, 0.5 mm; I, 0.3 mm; L, M, 0.25 mm. C, J, N, O: scale not provided by the author. Redraw from A: Mohammad, 1980; B: Fauvel, 1929; C: Gravier, 1904; D, M: Magalhães & Bailey-Brock, 2012; E, F, H: Green, 2002; G: Gillet, 1986; I: Reish, 1959; J: Hartman-Schröder, 1979; K: Jones, 1961; L: Silva, 1965; N: Cantone, 1976; O: Day, 1965; P: Doyle, 1991; Q: Rangarajan, 1963.

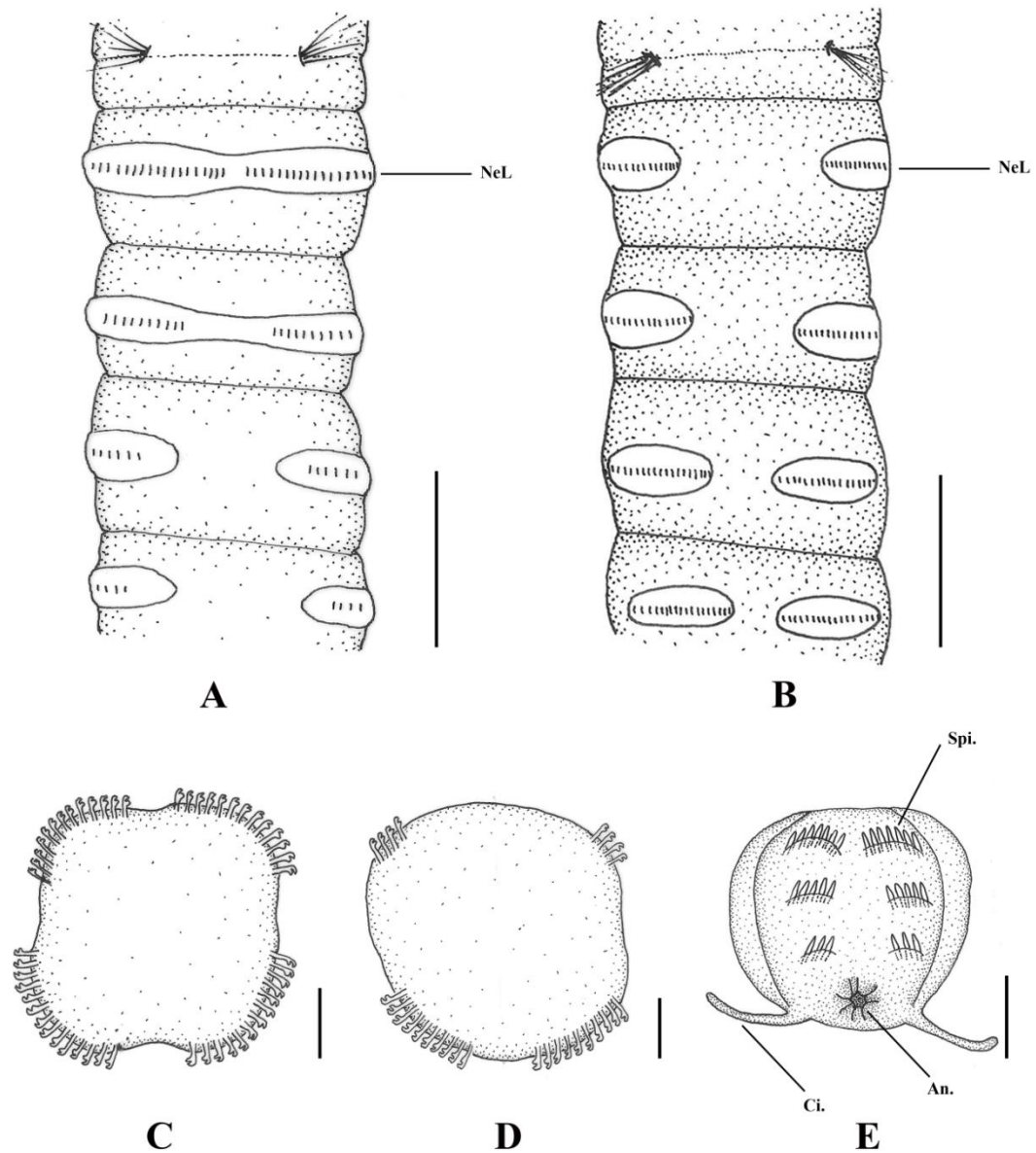


**Fig. 2.** *Scyphoproctus A* sp. n. (A) Thoracic region, dorso-lateral view; (B) Thoracic region, dorso-lateral view, methyl green staining pattern; (C) Abdominal region; dorsal view; (D) Abdominal region; ventral view; (E) Thoracic and abdominal region; lateral view, methyl green staining pattern; (F) Anal plaque, frontal view; (G) Anal plaque, lateral view; (H) Anterior end, dorso-lateral view, methyl green staining pattern; (I) Abdominal region, ventral view, methyl green staining pattern. AS: achaetous segment. Pe: peristomium. Pr: prostomium. Ch: chaetiger. T: thorax. A: abdomen. NoL: notopodial lobe. NeL: neuropodial lobe. LO: lateral organ. An: anus. Ci: cirri. Scale bars: A, D, H, I, 0.5 mm; B, 1 mm; C, E, F, 0.2 mm; G, 0.1 mm.

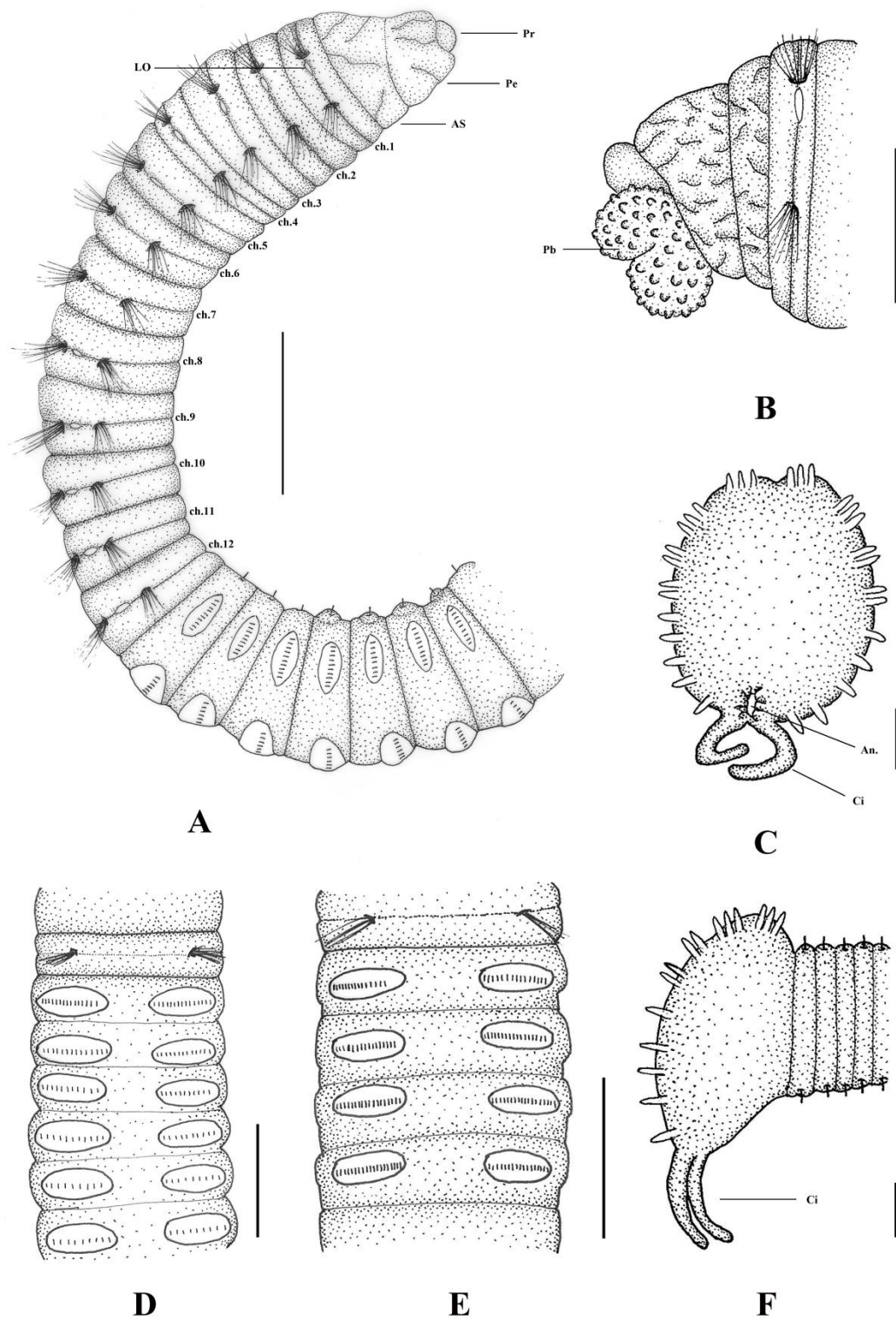


**Fig. 3.** *Scyphoproctus A* sp. n., SEM. (A) Thoracic region, dorso-lateral view; (B) Anterior notopodial hooded hook, frontal view; (C) Anal plaque, lateral view; (D) Anal plaque, frontal view; (E) Posterior notopodial hooded hooks, top view; (F) Anterior neuropodial hooded hooks, frontal view; (G) Posterior neuropodial hooded hooks, frontal view. AS: achaetous segment. Ch: chaetiger. Pe: peristomium. Pr: prostomium. LO: lateral organ. NoL: notopodial lobe. NeL: neuropodial lobe. An: anus. Ci: cirri. Spi: spines. Scale bars: A, 0.5 mm; B, 2 μm; C, D, 0.1 mm; E, F, G, 5 μm.

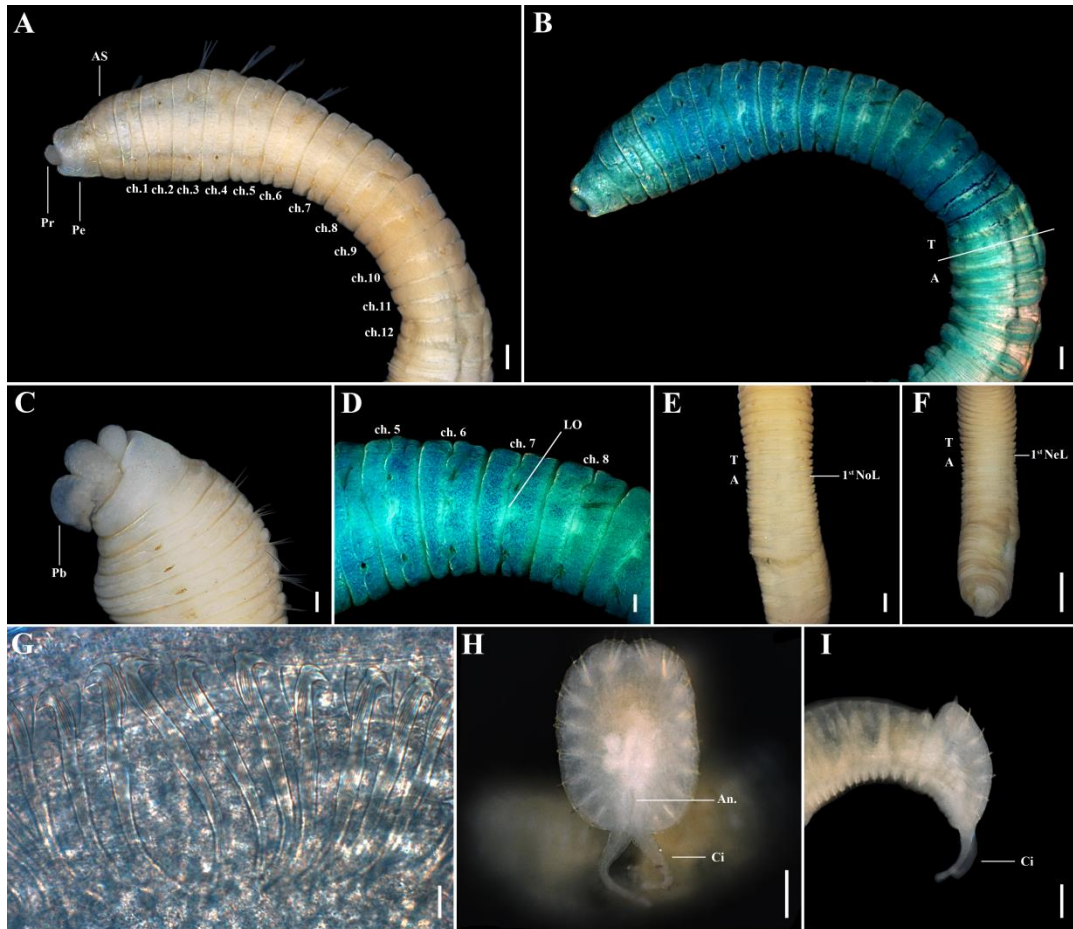




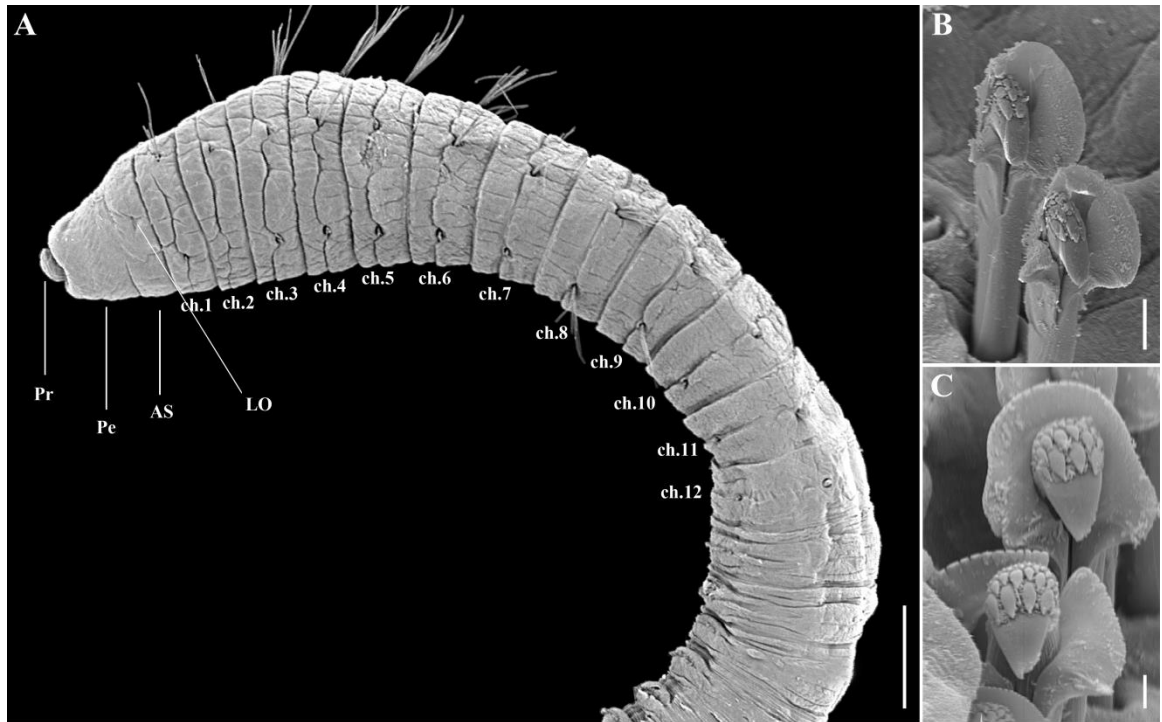
**Fig. 4.** *Scyphoproctus* A sp. n. (A) Abdominal region; dorsal view; (B) Abdominal region; ventral view; (C) Anterior abdominal chaetiger; (D) Posterior abdominal chaetiger; (E) Anal plaque, frontal view. NoL: notopodial lobe. NeL: neuropodial lobe. An: anus. Ci: cirri. Scale bars: A, B, E, 0.5 mm; C, D, 0.2 mm.



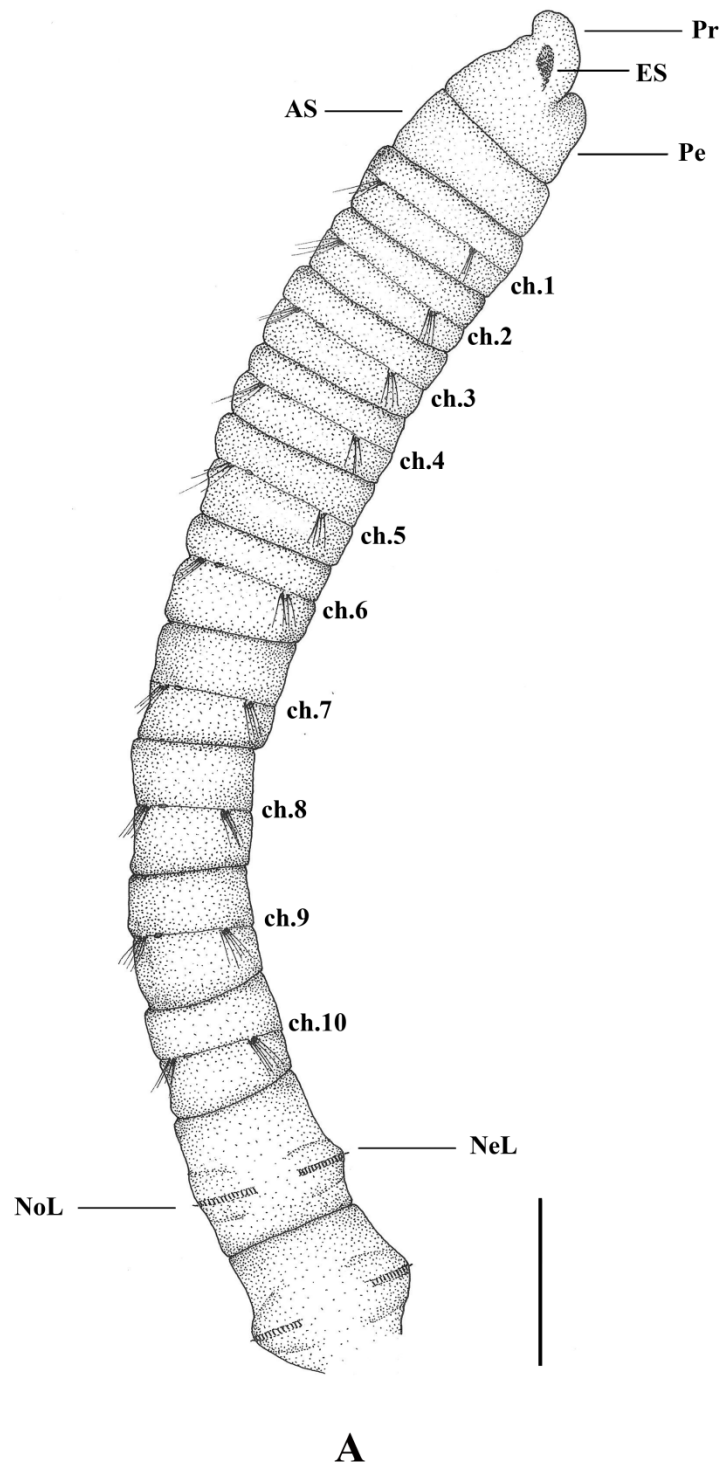
**Fig. 5.** *Scyphoproctus B* sp. n. (A) Thoracic region, lateral view; (B) Anterior end, lateral view; (C) Anal plaque, frontal view; (D) Abdominal region; dorsal view; (E) Abdominal region; ventral view; (F) Anal plaque, lateral view. AS: achaetous segment. Pb: propocis. Pe: peristomium. Pr: prostomium. LO: lateral organ. An: anus. Ci: cirri. Ch: chaetiger. Scale bars: A, 1 mm; B, D, E, 0.5 mm; C, F, 0.1 mm.



**Fig. 6.** *Scyphoproctus B* sp. n. (A) Thoracic region, lateral view; (B) methyl green staining pattern; (C) Anterior end, lateral view; (D) lateral organs; Anal plaque, frontal view; (E) Abdominal region; dorsal view; (F) Abdominal region; ventral view; (G) Neuropodial hooded hooks, lateral view; (H) Anal plaque, frontal view; (I) Anal plaque, lateral view. AS: achaetous segment. Pb: propocis. Pe: peristomium. Pr: prostomium. LO: lateral organ. Ch: chaetiger. An: anus. Ci: cirri. T: thorax. A: abdomen. NoL: notopodial lobe. NeL: neuropodial lobe. Scale bars: A, B, E, 0.2 mm; F, 0.5 mm; C, D, H, I, 0.1 mm; G, 10 µm.

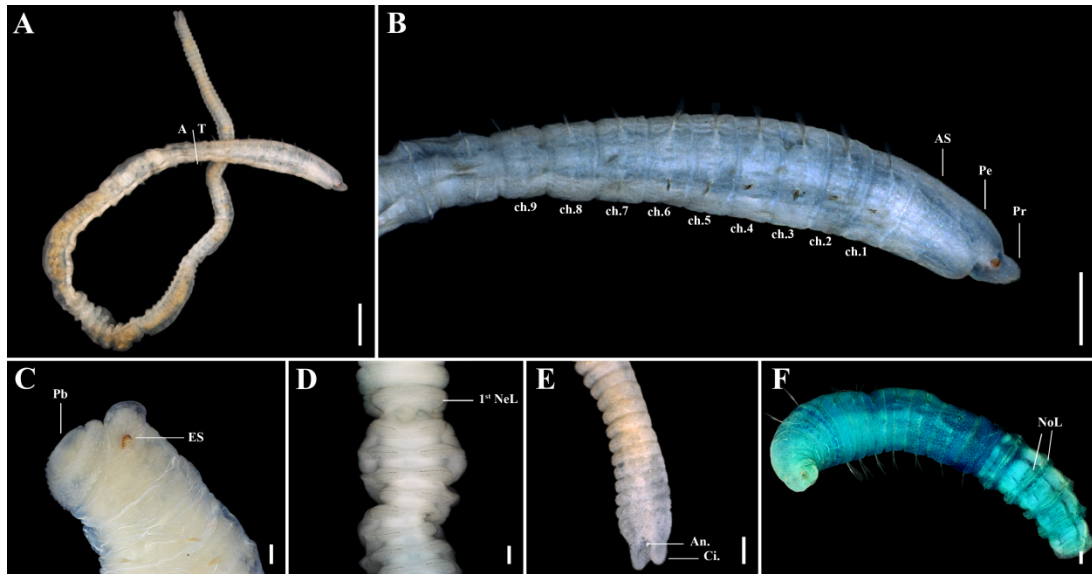


**Fig. 7.** *Scyphoproctus B* sp. n., SEM. (A) Thoracic region, lateral view; (B) Notopodial hooded hooks, frontal view; (C) Neuropodial hooded hooks, frontal view. AS: achaetous segment. Ch: chaetiger. Pe: peristomium. Pr: prostomium. LO: lateral organ. Scale bars: A, 0.5 mm; B, 5 µm; C, 2 µm.



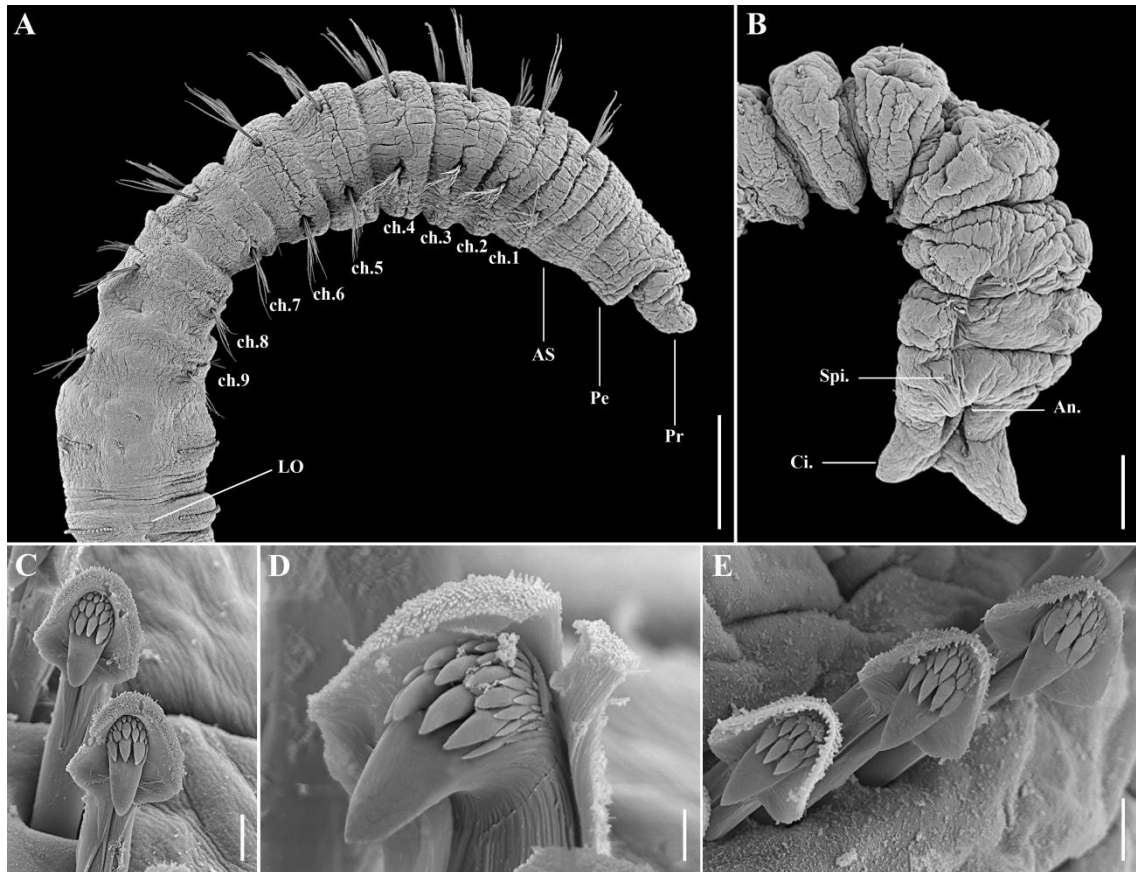
**Fig. 8.** *Scyphoproctus C* sp. n. (A) Thoracic region, lateral view. AS: achaetous segment. Pe: peristomium. Pr: prostomium. Ch: chaetiger. ES: eyespots. NeL: neuropodial lobe. NoL: notopodial lobe. Scale bar: A, 1 mm.





**Fig. 9.** *Scyphoproctus C* sp. n. (A) Complete specimen, lateral view; (B) Thoracic region, lateral view; (C) Anterior end, lateral view; (D) Abdominal region, ventral view; (E) Posterior end; ventral view; (F) Incomplete specimen, methyl green staining pattern. T: thorax. A: abdomen. AS: achaetous segment. Pe: peristomium. Pr: prostomium. Ch: chaetiger. Pb: propocis. ES: eyespots. NeL: neuropodial lobe. An: anus. Ci: cirri. NoL: notopodial lobe. Scale bars: A, 1 mm; B, 0.5 mm; C, D, E, F, 0.2 mm.





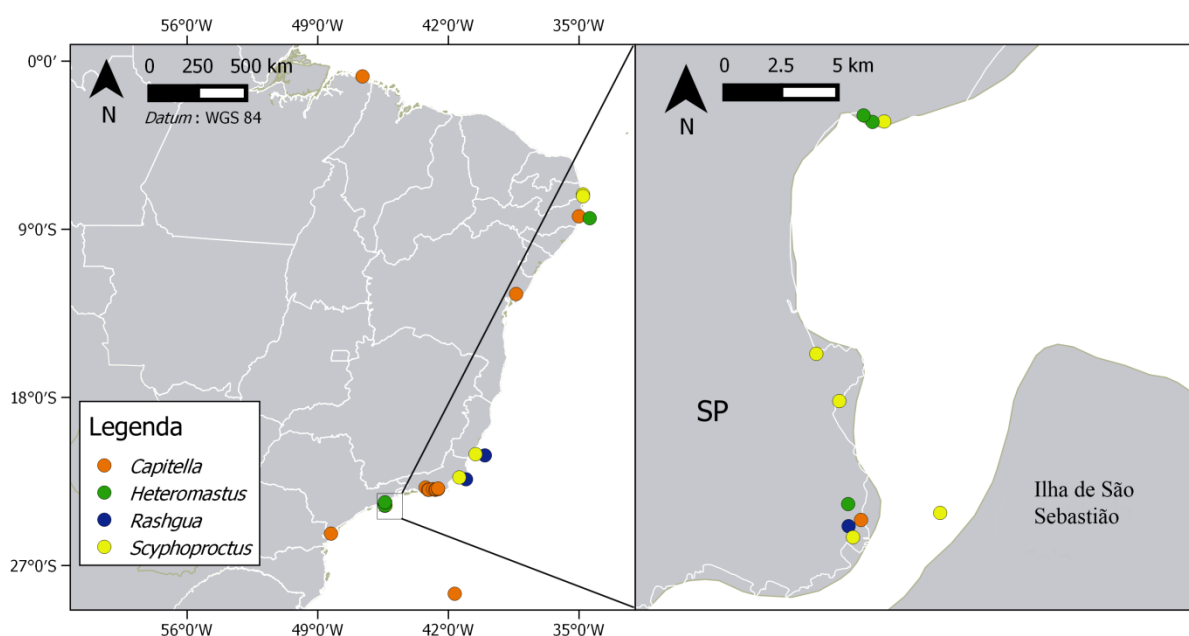
**Fig. 10.** *Scyphoproctus C* sp. n., SEM. (A) Thoracic region, lateral view; (B) Posterior end, ventral view; (C) Notopodial hooded hooks, frontal view; (D) Notopodial hooded hooks, lateral view; (E) Neuropodial hooded hooks, frontal view. AS: achaetous segment. Ch: chaetiger. Pe: peristomium. Pr: prostomium. LO: lateral organ. An: anus. Ci: cirri. Spi: spines. Scale bars: A, 0.5 mm; B, 0.2 mm; C, E, 5 µm; D, 2 µm.

## CONSIDERAÇÕES FINAIS

Nesse estudo foi examinado um total de 12 espécies, sendo 11 novas para a ciência.

Até o momento, tínhamos apenas um registro de Capitellidae em profundidade (1126 m) no Brasil, realizado por Attolini (2002) na Bacia de Campos (RJ e ES). Com a descrição de *Capitella iatapiuna* em ossos de baleia, foi possível expandir esse registro para regiões abissais (4204 m). As demais espécies de *Capitella*, de *Heteromastus* e *Scyphoproctus* sp. n. C foram registradas principalmente em regiões entremarés, não excedendo 0,5 m de profundidade.

Os diferentes gêneros identificados ocorreram ao longo das 23 localidades de coleta. No entanto, podemos observar que todos esses gêneros, e nove das 12 espécies identificadas, ocorreram na Baía do Araçá (Fig. 1). Localizada no litoral norte do estado de São Paulo, essa pequena baía apresenta heterogeneidade sedimentológica e condições oceanográficas propícias a uma rica diversidade biológica (Amaral et al., 2016). Inclusive, uma das espécies novas, *Capitella aracaensis*, é aqui reconhecida endêmica da Baía do Araçá.



**Fig 1.** Distribuição dos diferentes gêneros de Capitellidae coletados.

Por serem organismos com uma morfologia muito simples, a taxonomia dos Capitellidae não é trivial e sim relativamente mais trabalhosa, criando-se assim, um tabu com a identificação dos mesmos. Com o objetivo de facilitar futuras identificações, consideramos que uma das principais contribuições desse trabalho foi a composição de tabelas comparativas e chaves taxonômicas abordando todas as espécies válidas, visto que chaves taxonômicas locais podem acarretar em erros de identificação. Além disso, quando necessário, a diagnose do gênero foi modificada para acomodar variações dos caracteres ou inclusão de novos.

Apesar de simples, as espécies puderam ser diferenciadas, com êxito, por meio da taxonomia morfológica. No caso do gênero *Capitella*, os caracteres utilizados foram olhos, formato e tamanho do prostômio e peristômio, a formação do peristômio como um anel completo ou incompleto, número e distribuição de cerdas capilares e ganchos encapuzados ao longo do tórax, detalhes dos ganchos genitais, número, tamanho e estrutura dos ganchos encapuzados e formato e tamanho do pigídio.

No capítulo 2 foi apresentada a descrição de quatro espécies novas, anteriormente identificadas como sendo do complexo *Capitella capitata*, resultando na diminuição do cosmopolitismo generalizado. O número total de indivíduos identificados foi maior, em todos os locais de coleta, para *C. nonatoi* (3,507) seguido de *C. neoaciculata* (734), *C. biota* (149) e *C. aracaensis* (33). É bem provável que a espécie usualmente chamada de *C. capitata* para o Brasil seja a *C. nonatoi*, devido à sua discrepante abundância e ampla distribuição geográfica (Pará – Paraná). Assim, recomendamos atenção a esse novo desdobramento do complexo *C. capitata* em futuras identificações.

Em relação ao gênero *Heteromastus*, uma tabela comparativa foi fornecida e sua diagnose modificada com relação ao tamanho do prostômio, presença de cirro caudal e alteração da nomenclatura de *brânquias* para *expansões parapodiais*, uma vez que os Capitellidae não têm sistema circulatório e essas projeções da parede do corpo contêm extensões celômicas ao invés de *loops* do sistema circulatório (Fauchald & Rouse, 1997).

Espécimes do gênero *Rashgua* foram identificados como *Notomastus* por muito tempo por apresentarem o mesmo número de setígeros torácicos (11), assim como em muitos outros gêneros, porém é o único em que a maioria dos ganchos notopodiais do abdômen é ausente. A diagnose desse gênero foi modificada para incluir caracteres das novas espécies descritas nesta tese.

Os capitelídeos do gênero *Scyphoproctus* são facilmente identificados pela distinta região pigidial. Esta geralmente compreende uma placa anal dorsalmente achatada, que pode

ser bem ou mal desenvolvida, consistindo de um número de segmentos fundidos com o pigídio e com espinhos aciculares. *Scyphoproctus* é um dos gêneros que apresenta variação no número de segmentos torácicos dentro da mesma espécie, dificultando sua identificação. Neste trabalho são fornecidas chave de identificação para todas as espécies válidas baseada na placa anal, que se mostrou ser um ótimo caractere para diferenciação das mesmas, e prancha com as placas anais para auxiliar na identificação.

Com o intuito de contribuir para a sistemática dos Capitellidae, pretende-se dar início ao estudo da filogenia morfológica do grupo, uma vez que trabalhos dessa natureza ainda são inexistentes. Adicionalmente, pretende-se dar continuidade às identificações dos Capitellidae de profundidade do Espírito Santo e Rio de Janeiro (Plataforma Continental, Talude e cânions).

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# ANEXO 1

18/04/2017

Gmail - Re: Enquiry: Paper published Deep Sea Res. I



Camila Fernanda Silva &lt;fercamis@gmail.com&gt;

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## Re: Enquiry: Paper published Deep Sea Res. I

1 mensagem

**Priede, Professor Imants G.** <i.g.priede@abdn.ac.uk>  
 Para: "fercamis@gmail.com" <fercamis@gmail.com>

18 de abril de 2017 04:23

Dear Ms. Silva,

My apologies for my failure to reply to your earlier enquiry. There is no problem with including your published paper in your PhD thesis. This is normal recommended practice for theses.

Best Wishes for success in your PhD.

Prof. Imants (Monty) G. Priede DSc FRSE  
 Deep-Sea Research I. Editor-in-Chief  
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<http://www.oceanlab.abdn.ac.uk/>

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**From:** [fercamis@gmail.com](mailto:fercamis@gmail.com) <fercamis@gmail.com>  
**Sent:** 17 April 2017 18:22:12  
**To:** Priede, Professor Imants G.  
**Subject:** Enquiry: Paper published Deep Sea Res. I

The following enquiry was sent via the Elsevier Journal website:

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 First Name: Camila  
 Last Name: Silva  
 Email: [fercamis@gmail.com](mailto:fercamis@gmail.com)

-- Message --  
 Dear Dr. Priede,

## ANEXO 2

15/05/2017

Gmail - Author Query to Editor PONE-D-16-48861R2 [ ref:\_00DU0lfis.\_5000BbpQGf:ref ]



Camila Fernanda Silva &lt;fercamis@gmail.com&gt;

### Author Query to Editor PONE-D-16-48861R2 [ ref:\_00DU0lfis.\_5000BbpQGf:ref ]

1 mensagem

**plosone** <plosone@plos.org>  
 Para: "fercamis@gmail.com" <fercamis@gmail.com>

15 de maio de 2017 14:25

Dear Dr Silva,

Thank you for your email

It is fine for you to make this content available as a doctoral thesis.

I hope this is of assistance and look forward to hearing from you if you require our further assistance at this time.

Kind regards,

Louise Franklin  
 EO Staff  
 PLOS ONE

Case Number: 05205698

----- Original Message -----

From: Camila F. Silva [[em@editorialmanager.com](mailto:em@editorialmanager.com)]  
 Sent: 5/13/2017  
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Manuscript information: PONE-D-16-48861R2

=====

PONE-D-16-48861R2

Demystifying the Capitella capitata complex (Annelida, Capitellidae) diversity by morphological and molecular data along the Brazilian coast

PloS One

=====

Dear editor,

My PhD is about the taxonomy of the family Capitellidae (Annelida) and this paper is part of the development of my study. I'd be very glad if I could include it in my thesis.

So I'm asking a permission to include it in my doctorate thesis.

Thank you so much.  
 Camila Silva.

Kind Regards,

Louise Franklin  
 EO Staff  
 PLOS ONE  
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## ANEXO 3



COORDENADORIA DE PÓS-GRADUAÇÃO  
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### DECLARAÇÃO

Em observância ao §5º do Artigo 1º da Informação CCPG-UNICAMP/001/15, referente a Bioética e Biossegurança, declaro que o conteúdo de minha Tese de Doutorado, intitulada "***Descobrimos novos Capitellidae (Annelida) do Brasil***", desenvolvida no Programa de Pós-Graduação em Ecologia do Instituto de Biologia da Unicamp, não versa sobre pesquisa envolvendo seres humanos, animais ou temas afetos a Biossegurança.

Assinatura: \_\_\_\_\_

Nome do(a) aluno(a): Camila Fernanda da Silva

Assinatura: \_\_\_\_\_

Nome do(a) orientador(a): Antonia Cecília Zacagnini Amaral

Data: 18/08/2017

## ANEXO 4

### Declaração

As cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que constam da minha Dissertação/Tese de Mestrado/Doutorado, intitulada **Descobrimos novos Capitellidae (Annelida) do Brasil**, não infringem os dispositivos da Lei n.º 9.610/98, nem o direito autoral de qualquer editora.

Campinas, 18/08/2017

Assinatura : \_\_\_\_\_

Nome do(a) autor(a): **Camila Fernanda da Silva**

RG n.º 45.697.822-7

Assinatura : \_\_\_\_\_

Nome do(a) orientador(a): **Antonia Cecilia Zacagnini Amaral**

RG n.º 4.153.623